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Arms talks: a new objective

ARMS-control talks, particularly those aimed at strategic arms limitation (SALT) and mutual and balanced forced reductions (MBFR), use as their primary instrument quantitative restrictions on numbers of men and weapons. This tool 'no longer fits the task', argues Christoph Bertram, Director of the International Institute for Strategic Studies in a just-published paper*. Moreover, 'unless we can develop a better tool, the task itself may fall into disrepute'.

Bertram proposes a major shift away from quantitative and towards qualitative measures. Counting weapons and men is often the outward sign, in arms-control agreements, of an implicit understanding among the participants of the military missions or tasks which they are trying to proscribe. Why, then, not make perfectly explicit what is meant to be stopped? A qualitative statement, such as that the participants have agreed not to pursue a first-strike capability is not tied to particular hardware and is independent of the rapid technological change that threatens many current agreements.

Bertram begins by looking at the dilemmas of present arms-control discussions. They have to be seen politically as fair and equitable, and this is an almost impossible task given the complex of asymmetries both in doctrine and hardware which goes to make up the strategic balance, and given the technological developments which soon render any agreement obsolete. So quantitative agreements soon attract political obloquy. They are also getting more and more difficult to verify, as new developments are largely in electronics which need never be spotted from satellites, and as field testing can often be dispensed with. Finally, new weapon developments often do not fit easily into old categories such as nuclear and non-nuclear, offensive and defensive, strategic and tactical. The cruise missile is one such weapon which has caused many problems in arms-control discussions.

It is possible to argue that agreements like SALT confer few benefits and that we should back off from giving arms control as practised at present the priority it has had. Certainly there are alternatives: we could try to slow the pace of technological development, either by putting ceilings on R&D expenditure or by more detailed measures such as limits on testing or on procurement. We could put a greater trust in unilateral measures which might generate more goodwill than is

generally evident at negotiating tables. But many of these measures have been tried in various forms in the past without singular success. We should look instead, says Bertram, at one arms-control agreement of recent years which has been effective, and learn from it: the 1972 Soviet-American agreement on Anti-Ballistic Missiles (ABM). This agreement is primarily a qualitative one aimed at preventing a particular military mission, the erecting of ballistic missile defences. The wording of the treaty makes it clear that the restriction does not just apply to the sort of ABM systems that were around in 1972; it applies to any system that might be dreamt up in the future. By not venturing into much detail on hardware, the ABM treaty also circumvents certain verification problems: within the SALT agreement one launcher too many constitutes a violation of a treaty; within the confines of the ABM treaty such infringements do not constitute an immediate threat but can be discussed in a more leisurely way.

What, then, would be the sorts of mission that new qualitative agreements might proscribe? Bertram suggests that in the strategic nuclear field they could include acquiring a first-strike capability, conducting strategic anti-submarine warfare and developing anti-satellite capabilities. In other fields they might include possessing the ability to launch a massive surprise attack in Europe or cutting vital supply lines at sea.

Quantitative negotiations would not come to an end if this new style of agreement were to become the accepted pattern, but detailed balancing would not occupy the centre of the stage. It would be up to participants to prepare their own appropriate mixture of arms constraints consistent with the intent of the agreement and if necessary to defend the mixture against challenge by other participants. The nature of the mixture could always change with time, provided it continued to satisfy all parties to an agreement.

The ideas that Bertram proposes are, by his own admission, not radically new in that they are already incorporated in the ABM Treaty and some multilateral treaties such as that on biological weapons. But they have never been explicitly stated before in connection with the major East-West agreements now under review. They come at a time when there is a climate of opinion emerging which is dissatisfied with the way SALT is proceeding, particularly with the detailed complexities in which it is perennially bogged down. Qualitative arms control agreements wouldn't eliminate these complexities but negotiators would know that the real priority lay in the statement of intent. □

**The Future of Arms Control: Part II Arms Control and Technological Change: Elements of a New Approach. Adelphi Paper 146, International Institute for Strategic Studies.*



Peer review clash in US agricultural research

The Carter administration is locked in a fierce struggle with members of Congress over the most effective way of financing basic research in the agricultural sciences.

The latest round took place last week, when the Senate agreed to restore to the Department of Agriculture's 1979 budget \$30 million which the administration had requested for a system of competitive research grants, but which had been rejected by the House of Representatives.

The administration's moves follow a number of reports which have appeared in recent years emphasising that quality of agricultural research can only be maintained by putting grant applications on to the conventional competitive basis used in other areas.

Opposition from members of Congress has reflected the fears of the numerous state colleges and research institutions which, under the Hatch Act of 1887, are at present allotted money for research purely on the basis of farm population and outputs.

Last year, the House cut \$16.7 million off an administration request for \$26.7 million for a competitive grants system; however, the full amount was restored by the Senate, and a compromise figure of \$15 million was eventually agreed upon.

The competitive grants programme is now under way, and following requests sent out by the Agriculture Department's Science and Education Department in February, over 1,000 research applications have been received for universities and colleges throughout the country—the total funding requested being over \$200 million.

Yet despite the popularity of the scheme in the scientific community, opposition remains high. And last month, particularly concerned at the administration's proposal to reduce

Hatch Act research "as part of a re-orientation of research priorities" from \$109 million to \$98 million, the House not only voted to restore this cut, but also to delete the full \$30 million which had been requested for competitive grants.

In giving reasons for the deletion, Representative Jamie Whitten, chairman of the house agriculture appropriations subcommittee, took a swipe at the peer-review system: "Congress must not allow itself to be placed in the position of being held accountable to the people for the research priorities established by a non-elected bureaucrat issuing grants to his fellow scientists."

And in order to rub the message in, the House included in the appropriations bill a detailed list of individually-defined research grants, ranging from \$10 million for animal and health plant research, to \$25,000 for dried bean research.

The move provoked an angry reaction from the administration. Appearing before the Senate appropriations subcommittee, Agriculture Secretary

Bob Bergland accused Mr Whitten of "seriously impairing" his right to run the department; and President Carter sent a personal letter to the subcommittee chairman, Senator Thomas Eagleton, asking him to restore the competitive grants scheme.

This was dutifully done by the subcommittee, and approved by the whole Senate last week, which also deleted the provisions added by the House for specific research topics.

During the debate, for example, the Senate accepted an amendment from Senator John Melcher of Montana adding \$20 million for animal health and disease research. Senator Melcher claimed that many states were under-utilising the laboratory facilities and personnel existing in veterinary colleges and agricultural experiments stations; Senator Eagleton, in opposing the amendment, pointed out that the Department of Agriculture already plans to spend \$47 million on research in this area in 1979, and feels this to be "sufficient to address the priority needs" with no compelling reason to add more funds.

Responding to this and other amendments which would have added over \$500 million to the administration's request, the Senate subsequently approved a further amendment from Mr Eagleton cutting Hatch Act grants—which the Senate had previously increased from a requested \$98 million to \$113.9 million—by \$9.5 million to \$104.4 million. With honours now roughly even, the next round in the debate will take place in the conference between House of Senate. It is generally expected that about \$15 million—the same level as the current year—will be agreed for the competitive grants programme; but even this outcome will leave many far from satisfied.

David Dickson



"Gentlemen, I think it's time we diversified into peanut research!"

Solar orbital satellite launched

THE third and final satellite of the International Sun Earth Explorer, the US National Aeronautics and Space Administration's ISEE-C, was successfully launched on schedule from Cape Canaveral last Saturday. It is heading for an orbit around the Sun at a distance of 1.5 million km from Earth. In this orbit it will observe phenomena in interplanetary space, and particularly the solar wind.

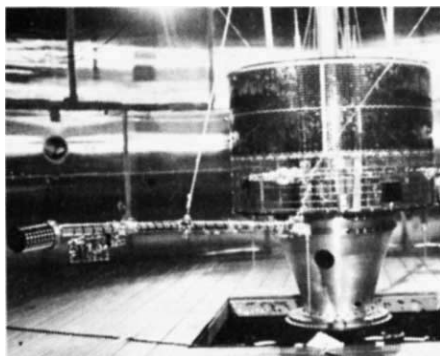
The other two satellites in the ISEE programme, NASA's ISEE-A and ISEE-B built by the European Space Agency, were launched in tandem by NASA from a Delta 2914 rocket last October. They were both put into the same highly elliptical Earth orbit with

closest and furthest points from the Earth of 280 km and 140,000 km respectively. Their task is to provide information on phenomena in the Earth's magnetosphere. Their highly elliptical orbit was chosen so that the satellites will make as many crossings as possible of the boundary where the solar wind meets the magnetosphere. Observation of this boundary could provide information on the magnetic confinement of plasma and the interaction of ionised particles with the Earth's magnetic field.

ISEE-C has a dual function: to investigate interplanetary space and the solar wind undisturbed by the Earth and to act as a reference satellite for

ISEE-A and B. The satellite has been built by NASA but European scientists are participating in some experiments. Teams from Imperial College, London, Utrecht in the Netherlands and ESA's Space Science Department are co-operating on an investigation of protons in interplanetary space; a team from Garching, West Germany is doing a study of the composition of solar particles and a team from Meudon, France is conducting a study of magnetic field lines emanating from the Sun.

The three satellites have been planned to make major contributions to the International Magnetospheric Study running from 1976-79. □



Geos 2 in space collision?

ESA's geostationary magnetospheric observatory satellite Geos 2 (above) appears to have suffered a collision in space. While the performance of much of the payload is unaffected, experiments studying the low energy plasma have been badly disturbed.

Geos 2, a replacement for Geos 1 which was lost in an aborted launch last year, became fully operational on 2 August. With all the onboard instruments functioning well, the prospects for a full two-year mission were excellent until 07.13 GMT on 5 August, when there was a temporary loss of UHF telemetry. The fault proved to be serious for the low energy plasma experiments, as it now seems that the electrical reference potential of the spacecraft is jumping by 12 volts for half of each 6-second spin cycle. It appears that damage to a small section of one solar array panel has resulted in the output of a string of cells shorting to the structure whenever they become illuminated.

There is now a whole catalogue of anomalies seen on high altitude satellites. It is fashionable to assume that they result from spacecraft charging events but, in the case of Geos 2 there are good reasons for looking elsewhere. Geos was carefully designed to eliminate differential charging: the solar cells have a conductive indium oxide coating and indeed this could provide the supposed shorting path if severe mechanical damage was experienced.

Well over a hundred spacecraft are now in geostationary orbit but there is no account of how many sensor covers, boom retaining clamps, attachment rings etc. have been ejected. Unlike cans thrown from cars along the road, they are sure to bounce back.

In the future, space shuttle may provide the needed 'vacuum cleaner' but in the meantime the warning is clear. The Geostationary corridor is filling rapidly and spacecraft designers must become litter conscious or risk the consequences.

Gordon Wrenn

UNCSTD: a ray of hope despite delay in planning



AN unexpected, faint, note of optimism was apparent in recent talks with Guy Gresford, Deputy to José da Costa, the Secretary General of the UN Conference on Science and Technology for Development (UNCSTD). This was surprising, because Gresford had just heard of the postponement by the Economic and Social Council (ECOSOC) of the third Preparatory Committee meeting of the Conference, from 18 September, to January 1979. So that fourth and final preparatory meeting, scheduled for next February, will now take place in May, only three months before the Conference itself. The wise arrangement of leaving six clear months for the final Conference preparations goes by the board: scarcely three months will now be available for the diplomatic negotiations with governments, particularly the "Group of 77" (now in fact 114) which are essential if UNCSTD is to be anything more than just another political confrontation.

It was the "77" who used their voting majority to obtain the postponement at the recent ECOSOC meeting in Geneva. Their reasons are not quite clear. One was allegedly the short time between the Conference on Technical Cooperation between Developing Countries (TCDC) in Buenos Aires later this month, and the September meeting. More likely, perhaps, is a feeling of frustration at the lack of any output from the Conference Secretariat worth discussing in September, and a general disillusion of the "77" (in addition to that earlier felt by other groups) at the performance of da Costa himself as Secretary General. In sharp contrast to Maurice Strong during the preparations for the Stockholm Environment Conference, da Costa has visited few member states, and been seen in few developing countries. Indeed, he has seldom apparently been seen around his own secretariat in New York, living as he has been mostly in Paris.

From the first, da Costa made it clear that the material for his Conference should come from member governments, especially (but perhaps optimistically) from the "77". But most of the latter need some guidance as to what is expected of them—something to chew on before they commit themselves. This has been notably lacking. It could have come from the UN Specialised Agencies, but these da Costa antagonised almost from the start. It might have come from within UN itself, but his refusal

to work with the Office for Science and Technology in New York made that impossible. But quite apart from this, the fact is that the whole UN system has become grossly overloaded by the ever-increasing number of meetings and conferences to be serviced, against continuing demands for economy and reduction of staff. One European delegate remarked that he would spend 40 of the next 52 weeks attending meetings of one sort or another: that is bad enough for a big delegation, but for countries that can barely raise more than a single representative even for an important meeting, it is intolerable.

Paradoxically, this very delay may add a little more to Gresford's cautious optimism, by giving more time to prepare for the next preparatory meeting, and in particular, to study the national papers which are the basic material for the main Conference background documents. Some 90 national reports had been received by the 1 August deadline, with presumably a few stragglers to come in. It is the quality, as well as the number, of those from the developing countries that have brought a ray of cheer to the hard-pressed secretariat; in particular, several of the least-developed and poorest countries have produced good papers—perhaps because they are among those to whom the services of over 50 special advisers have been provided in one way or another. This should make it easier to prepare the Action Plan for the Conference, always provided that governments will support, at the diplomatic level next year, what they have said in their pre-conference submissions. It is on this that the success or failure of the Conference depends, since the aim all along has been to avoid confrontation between North and South—in effect, between the "77" and rest—by having the recommendations and Action Plan agreed in advance, leaving the meeting itself for political speeches.

Meanwhile, the effect of the postponement will be felt all along the line. What is unlikely, however, is that the Conference itself will be postponed. As the first really big event held under Kurt Waldheim's Secretary Generalship in his own country, it is a matter of prestige for him that it will go according to plan, and any change of dates seems out of the question, quite apart from what the Austrian Government might have to say about a request for postponement.

Peter Collins

Further protests planned at Seabrook nuclear plant

NEW protests are being planned at the controversial Seabrook nuclear power plant—on the coast of New Hampshire—following last week's decision by the Nuclear Regulatory Agency to withdraw a ban on further construction work.

The ban, imposed last month, followed criticisms that the Environmental Protection Agency had adopted incorrect procedures in approving the 2.3 megawatt power station's proposed cooling system, which will involve discharging hot water into the sea.

Environmental groups which had opposed the scheme on the grounds that it would seriously damage the local marine life, had claimed the NRC's decision to suspend construction as a "major victory".

However with a speed that took many opponents by surprise—a decision had not been expected until mid-September—the EPA announced on 4 August that it had reconsidered the proposed cooling system, and still considered that it complied with federal water pollution laws.

Six days later the NRC, which had previously voted 2-1 to suspend construction, voted unanimously that the EPA's approval eliminated the conditions which had led to the suspension, and that work at the site could therefore continue.

Opponents of the Seabrook plant, which has become a national symbol



for the antinuclear movement and attracted a 10,000-strong, four-day demonstration in June, claimed that the EPA's decision had been made "with unprecedented haste in a politically charged atmosphere". A procedural suit has already been filed against both the NRC and the EPA by an attorney representing the two antinuclear groups that put together the Clamshell Alliance of several protesting organisations.

The NRC's decision has been welcomed by the Public Service Company of New Hampshire, the utility company which has already spent \$400 million on construction work at the Seabrook site, and had to lay off 1,800 workers.

However members of the Clamshell alliance are already preparing new protest plans. "Many different groups within the Clamshell Alliance will demonstrate their opposition to the ruling through a series of non-violent civil disobedience actions", said one member. Possible actions could include both further sit-ins at the site, where 1,400 demonstrators were arrested last summer, and a blockade by small boats of the vessels used to transport the main reactor components. □

Shadow of '68 still hangs over Czech science

TEN years ago this month, the appearance of Soviet tanks in the streets of Prague brought the Dubcek spring to a sudden and violent end. As well as occupying such strategic points as the Central Telegraph Office and the editorial premises of leading newspapers, the "fraternal" invaders took particular care to take possession of the building of the Czechoslovak Academy of Sciences.

So important was the occupation of the academy to them that the officer, a first lieutenant, who commanded the platoon which "seized the . . . building and organised its security and defence", ordering out the rightful occupants with an abrupt message in Russian (see caption), was subsequently recommended for the order of the Red Star.

The physical, though temporary, occupation of the Academy was followed, in due course, by administrative measures curtailing its freedom. As part of the post-1968 "normalisation", new statues were imposed on the academy, depriving it of much of its former autonomy and downgrading the qualifications required of its leading officials.

From 1970 vice-chairmen no longer had to be full members of the academy, and the general secretary need not even be a member. The chairman of the academy Dr Frantisek Sorm was dismissed, and replaced by one of the vice-chairmen, Jaroslav Kozesnik. Two new vice-chairmen were nominated by the government, and a new general secretary, Karel Friml, appointed, who did not hold a postgraduate degree. Scientists working at the academy lost their security of employment; their contracts were limited to four years.

Annual statistical handbooks reveal that the early 1970s were black years for Czechoslovak learning. Numbers of new graduates steadily rose from 1,200 a year to 1,516 a year between 1966 and 1969, but then fell sharply to 906 in 1970 and 875 in 1977. The figure remained at around 1,100-1,200 for the next four years and only in 1976 fully recovered to 1,504.

It was at the academy level, however, that the new measures were most severely felt. Ideological purity was emphasised above academic prowess. The new director of the Institute of Nuclear Physics, Jaroslav Prochazka, expressed this trend in the now-notorious saying: "I would throw out even Einstein if his political views

Antibiotics may stay in US animal feed

THE US Food and Drug Administration announced last week that it is delaying—and may eventually drop—proposals published earlier in the year to limit the distribution of animal feed pre-mixes and medicated feeds containing the antibiotics penicillin, chlortetracycline and oxytetracycline.

The proposal would have limited the distribution of such feed-mixes to feed mills which had been specially approved, and to the order of a licensed veterinarian. It followed earlier announcements by the FDA commissioner that he intended to eliminate the use of antibiotics in animal feeds because of the possible danger of the transference of antibiotic resistance, concerns that have led to a similar ban in Britain and other European countries following the Swann Report of 1970.

However such moves have been consistently opposed by the pharmaceutical and agricultural industries, the former claiming that it would lead to substan-

tial loss of sales, the latter that the extra food necessary to compensate for the withdrawal of antibiotics would push up the price of agricultural products.

In last week's announcement, Dr Kennedy says that since the January proposals were published, a number of concerns have been expressed by the public which need to be evaluated further.

These concerns include the contention that the number, distribution and kinds of veterinarians available are not adequate to diagnose and write the prescriptions under the proposed requirement, and assertions that the proposal will unnecessarily restrict the practice of veterinary medicine because it will eliminate flexibility in the dosage levels available to be prescribed.

The commissioner says that because of these and other concerns, he is considering "whether the proposals should be withdrawn or revised to better address the public's concerns". □



Prague, August 1968. The Academy of Science was closed with this order: "I, First Lieutenant Yurii Aleksandrovich Orlov, representing the Warsaw Pact troops, order all workers and members of the Czechoslovak Academy of Sciences to cease work by 13.00 hours, 22 August, and to evacuate all premises of the Czechoslovak Academy of Sciences."

were not quite in order!" The same sentiment was expressed more formally in the official *Vestník* (Bulletin) of the academy that "the final appointment to the post of institute directors will be made according to the designated list and in close association with the Party organs".

In 1977, all academy members had to complete an affidavit relating not only to their own political reliability but that of their relatives and in-laws.

Scientists who failed to satisfy these political criteria were kept in work on contracts so short as to suggest scientific piecemeal—and often, ultimately, driven into premature retirement. By 1974, it would seem that the losses to science due to post-1968 repression and emigration were causing serious shortages of personnel.

At the May Plenum of the Central Committee of the Communist Party of Czechoslovakia, which laid considerable stress on the scientific-and-technological development of the national economy, a resolution was passed which appeared to extend forgiveness to the hundreds of scientists and technologists who had been excluded from professional work due to political "black marks" from 1968. The wording of the resolution stressed however, that this second chance was being offered only to those "who today recognize their mistakes and by their positive efforts are proving their relationship to our society and contribute to its development".

Not surprisingly, many of the bolder spirits ignored the offer. By 1975, reports were reaching the West that still tens of thousands of scientists and artist, were barred from professional activity, and that a new wave of police searches of the homes of former scientists was in full swing. New legislation, it was reported, would curtail the powers and responsibilities of university rectors and facilitate the dismissal of politically "unreliable" individuals.

The effect of such measures on Czechoslovak science has been considerable. During the 1970's Czechoslovak participation in international conferences has fallen off drastically and, although according to Comecon statistics, Czechoslovakia plays a major role in the joint nuclear research programme, in 1973 and 1974 there was no Czechoslovak representation at all at two international nuclear physics conferences.

Publication, even at home, is drastically controlled; publication in a Western country is often dependent on previously having published papers in a Soviet journal—a condition hard to fulfil in view of the lag in Soviet publication schedules.

Access to western journals is severely limited. According to Professor Frantisek Janouch, now working at the Stockholm Institute of Nuclear Physics, in 1974 some 14 issues of *Nature* were confiscated by the authorities ("withdrawal of the right to delivery" is the

official euphemism for this practice).

Not surprisingly, many former Czech scientists see these restrictions as disastrous. According to microbiologist Ivan Malek, prior to 1968 a member of the Central Committee of the Party, these measures "brought Czechoslovak science to its knees in recent years"; and are likely to bring about a state of "catastrophic backwardness". Even scientific cooperation within Comecon, he maintained, "which ought at least to redress the opportunities that have been spoiled, is not developing in conformity with the needs and the real character of science. It has not been able to free itself from heavy bureaucratic formalism."

Malek delivered that message in 1975. Since then Czechoslovak science has had what might be seen as one relieving factor in a vista of otherwise mediocrity—the participation of Vladimir Remek in the first "international" Comecon space-flight. Yet it is perhaps significant that the Czech media stressed throughout the "close cooperation" with the Soviet Union, and the fact that the Soviet Union bore some 95% of the cost. (The Poles, on the other hand greeted the flight of *their* representative by stressing the specifically Polish contribution.)

Nor is this emphasis on Soviet aid an isolated instance. Dr Eva Dubská, a psychiatrist, who emigrated from Czechoslovakia last year, reports that the peculiar theories of Academician Snehnevskii, the Soviet psychiatrist who attributes all mental illness (and for that matter, political opposition) to schizophrenia, are being increasingly introduced into Czech psychiatric theory.

After a decade of political pressure, it is not perhaps surprising that only slightly more than 10% of the original signatories of Charter 77 were scientists or technologists. Zdenek Mlynar, a prominent Chartist, told *Nature* last year that the majority of potential dissidents had long since been driven out of the Czechoslovak scientific establishment. The surprising thing, he implied, was that there were any left at all.

Nevertheless, the number of signatures by scientists, although considerably smaller than that of writers, artists and workers, is large enough to demonstrate that Czech science is still not entirely Party-subservient. Moreover the presence of at least two scientists, Vladimir Lastusek, a nuclear engineer, and Ales Machacek, an agronomist, in prison for having distributed copies of the charter, suggests that the 1968 occupation of the academy cannot be taken as entirely symbolic of the future of Czechoslovak science.

Vera Rich



Science and technology in Southeast Asia

A survey by Yongyuth Yuthavong

FIVE countries form the Association of Southeast Asian Nations (ASEAN): Indonesia, Malaysia, Philippines, Singapore and Thailand. They share many common features, including limited financial resources and a relatively brief acquaintance with science and technology.

With the exception of Singapore, all the ASEAN countries are agriculturally productive and endowed with rich natural resources. They share similar scientific and technological priorities, centred on the development of agriculture and the use of natural resources, while population, health and nutrition also present problems similar to those in other developing countries. Private industry in the area still has a small capacity and mostly involves low and medium levels of technology. Consequently most research and development activities are concentrated in the government sector. Science and technology policy in these countries therefore has a relatively greater impact than in the industrial countries where much activity is in the private sector and less subject to government control.

Without exception, research and development activities in ASEAN countries are heavily geared towards urgent socio-economic, food and health problems. This is understandable in view of the limited financial resources and the magnitude of these urgent problems. There are, however, differences in emphasis and detail depending on the resources of the individual countries. Hence, while the other four countries are preoccupied with problems in rural development, Singapore is mainly concerned with problems in industrial technology. Topics of high priority for research and development in the other four countries include agri-

culture, natural resources, small-scale industry and tropical health. Basic science plays only a minor role.

A substantial portion of research and development in the Philippines is supported by the National Science Development Board (NSDB) or done by its own agencies. The development of geothermal power by the Commission on Volcanology, of new ceramic products by the National Institute of Science and Technology and of low cost materials for housing by the Forest Products Research and Industries Commission are examples of such work. Research in universities includes plant breeding and related agricultural research at Central Luzon State University, and aquaculture at the College of Fisheries and the Marine Sciences Centre of the University of the Philippines.

Research of high priority in Indonesia concerns a comprehensive inventory and evaluation of natural resources, comprising land and water, vegetation, aquatic, energy and mineral resources. Biological research, including collection, survey and breeding, is carried out at the National Biological Institute at Bogor, founded in 1817, which has one of the largest tropical botanic gardens. The National Institute of Oceanology, with centres in Jakarta and Ambon, conducts and co-ordinates various fields of marine research. Other national institutes include those of geology and mining, chemistry, physics, metallurgy, electrotechniques and instrumentation, all located in Bandung.

Important research institutes in Malaysia include the Rubber Research Institute, the Agricultural and Research Development Institute, the Mining Research Institute, the Geological Survey Laboratory, the Standards and Industrial Research Institute of Malaysia, and the Institute for Medical Research. Unlike those in the Philippines and Indonesia, the institutes are not under central control but



belong to various ministries. Of the five universities in Malaysia, the University of Malaya in Kuala Lumpur and of Sains Malaysia in Penang have the potential for doing good scientific research.

The University of Singapore, which was originally a part of the University of Malaya, is also quite well established in various branches of science. In the field of mathematics Nanyang University in Singapore is outstanding, being the home of the famous Southeast Asian Mathematical Society.

In contrast to the other ASEAN countries, a relatively high proportion of research in Thailand is done at universities, especially in basic sciences. Some universities such as Mahidol University and King Mongkut Institute of Technology have research support from international sources in addition to local budget. Most applied research is done in research units of various departments, such as the Departments of Fisheries, Irrigation and Mineral Resources. Since 1963, the government also established the Applied Scientific Research Corporation of Thailand specifically to conduct applied research in selected projects.

Regional co-operation and assistance from international agencies have also influenced the research and development efforts of ASEAN countries. The International Rice Research Institute (IRRI) in the Philippines and the Asian Institute of Technology

Yongyuth Yuthavong of the Department of Biochemistry, Mahidol University, Bangkok, Thailand, has been a Nature travelling fellow.



The key subjects for research: food, health and use of natural resources



(AIT) in Thailand are two important international institutes founded on such bases. Established in 1960 in Los Banos at the same location as a campus for the University of the Philippines, IRRI found early fame in the development and release of IR 8 "miracle rice" and subsequently of other varieties of semidwarf rice with such genetic qualities as desirable agronomic characteristics, resistance to insects, diseases and drought, high nutritive values and tolerance to floods, extremes of temperature and other hostile environments. In its genetic evaluation and utilisation programme, IRRI has a germ plasm bank which holds about 40,000 samples of both old and newly developed varieties of rice.

Other research activities at IRRI cover the problems of cropping patterns, control and management of pests, irrigation and water management, post-production technology, development of appropriate small farming machinery, and development of mini-kits for extension services to the farmers. Formal academic training is conducted in co-operation with the College of Agriculture of the University of the Philippines.

The AIT, located in a suburb of Bangkok, is concerned with studies and research in various areas of engineering and development. It has support from some 20 governments and 40 other international donors. Research in water-resources engineering, for example,

concerns the basic hydrological patterns for Southeast Asia, coastal engineering, fluid mechanics, irrigation and drainage. Geotechnical engineering research deals with the problems of local clays and soils as constructional materials, surveying and evaluating land resources, the development of highway materials and constructional technology, and agricultural soil science. Low-cost housing technology, and management and control of the tropical environment, and industrial engineering are other areas of engineering research.

A few educational and research centres are supported through the programmes of the Southeast Asian Ministers of Education Organisation and Ministerial Conferences for the Economic Development of Southeast Asia, ASEAN Permanent Committees and Secretariat, UNESCO, UNDP and various other UN agencies (see box).

Philippines

The most forceful planning agency of the five ASEAN countries is perhaps the NSDB (National Science Development Board) of the Philippines. With a direct annual budget of about £6m and an additional £12m for research agencies under its supervision, NSDB formulates policy, sets research priorities, gives research grants to universities and other agencies and also conducts research in its own agencies. There are six research agencies directly under NSDB: National Institute of

Science and Technology, Forest Products Research and Industries Development Commission, Philippine Textile Research Institute, Philippine Inventors Commission, Food and Nutrition Research Institute and Commission on Volcanology.

The role of NSDB in conducting research in selected fields is subject to criticism as being unsuitable for an agency responsible for science and technology policy of the whole country, since there might be a conflict of interest. However, this role also serves to stimulate activities in the fields chosen to be of high priority. An area of 35.6 hectares of public land in Manila is being developed as the Philippine Science Community, containing the office of NSDB, its research agencies and such attached agencies as the National Research Council of the Philippines, a prestigious council of elected scientist members, and the Science Foundation of the Philippines, mainly concerned with the promotion of science consciousness of the public.

Indonesia

A rough equivalent of NSDB of the Philippines is the Office of the Minister of State for Research of Indonesia, which has a twofold task of assisting the president in formulating government policy for research and other scientific and technological activities, and in supervising the activities of its own agencies. These are the National Atomic Energy Agency, National Space and Aeronautics Agency, National Coordinating Agency for Surveying and Mapping, and Indonesian Institute of Sciences (IIS).

The IIS is the most important both in formulating science policy and in conducting research. Ten national institutes located in Jakarta, Bogor and Bandung are under its responsibility. Created in 1967 with the aim of eventually forming an Indonesian Academy of Sciences similar to the Academies of Sciences in continental Europe, the IIS with its double function, like NSDB of the Philippines, may be criticised for having a conflict of interests. The subsequent creation of the post of the Minister of State for Research formally transfers policy decisions one step further, but still leaves the task of underlying data collection and analysis with the Institute.

Malaysia

Science policy guidelines in Malaysia are outlined by the National Council for Scientific Research and Development, an advisory body to the government formed two years ago, the secretariat of which is in the Ministry for Science, Technology and Environment. The council has five committees dealing with agricultural science,

medical science, marine science, industrial science and technology, and socio-economy.

The Ministry has responsibility for such agencies as the Standards and Industrial Research Institute, the Tun Ismail Atomic Research Centre, the Chemistry Department and the Wildlife National Parks. The Rubber Research Institute, which is a big and successful institute dealing with development of the most important economic commodity of the country is not under this Ministry but under the Ministry of Primary Industries.

Singapore

Science policy guidelines in Singapore are drawn up by the Ministry for Science and Technology, a small ministry which also runs some service departments and has responsibility for promoting public appreciation of science and technology. For the last purpose an impressive Science Centre has been established, and a major part of the activities of the Science Council, an advisory body composed of the nation's scientific and technical leaders, is devoted to such popular television programmes as the science quiz. With the exception of the recent establishment of the Applied Research Corporation, however, the Ministry is not directly concerned with research and development activities. For example, the important Singapore Institute of Standards and Industrial Research is under the Ministry of Finance, and research in universities is supported mainly by the Ministry of Education.

Thailand

Thailand has no single organisation on science and technology of equivalent status to a ministry, but has the National Research Council (NRC) with the responsibility of advising the government on natural and social science policy, identifying and supporting research topics of high priority. It has five national committees covering the areas of physical science and mathematics, medical science, chemical and pharmaceutical science, agriculture and biology, and engineering and industrial research, and another five national committees for social science and humanities.

Formulation of Thai science and technology policy with special reference to economic development is also the task of a small division of the National Economic and Social Development Board. Having more than one agency in a small country dealing with science policy can be a source of potential conflicts, which fortunately have not materialised so far. Recently the NRC has recommended to the government that a Ministry of Science and Tech-

Regional co-operative programmes

A NUMBER of regional programmes in science and technology have been set up through the agreements among Southeast Asian countries. The following are a few of the important ones:

Southeast Asian Ministers of Education Organisation (SEAMEO) programmes involve the support of centres of excellence in various fields in the ASEAN countries. Of the six regional centres, five are concerned with science and technology. These are Regional Centre for Tropical Biology (BIOTROP) located in Bogor, Indonesia; Regional Centre for Educational Innovation and Technology (INNOTECH) in Quezon City, Philippines; Regional Centre for Graduate Study and Research in Agriculture (SEARCA) in Los Banos, Philippines; Regional Centre for Education in Science and Mathematics (RECSAM) in Penang, Malaysia; and Regional Project for Tropical Medicine and Public Health (TROPMED), which is a network of national centres with the head office in Bangkok, Thailand.

Ministerial Conferences for the Economic Development of Southeast Asia, usually attended by ministers of foreign affairs of member countries, which include Japan, Australia and New Zealand in addition to the ASEAN countries, support various programmes in science and technology. Important bodies in the group are Southeast Asian Fisheries Development Centre (SEAFDEC), Southeast Asian Agency for Regional

Transport and Communication Development (SEATAC) and Southeast Asian Medical and Health Organization (SEAMHO).

ASEAN has a Bureau of Science and Technology and Permanent Committees (PC) on various aspects of science and technology, namely, PC on Science and Technology, PC on Food and Agriculture, PC on Communication/Air Traffic Services and Meteorology, PC on Commerce and Industry and PC on Land Transportation and Telecommunications. An important project of the PC on Science and Technology is on soybean and other protein-rich foods funded with an aid of 2.5 million Australian dollars granted by Australia.

The Regional Office for Science and Technology for Southeast Asia of UNESCO actively supports various co-operative programmes. In the past few years with the support of UNESCO and other agencies, regional networks have been developed in specific areas of research, involving a number of national institutions or bodies linked together to facilitate regional communication and co-operation. The headquarters of the Regional Networks for Microbiology and for Chemistry of Natural Products are located in Bangkok, and for Geosciences in Seoul. A regional programme for postgraduate training and research in marine sciences for Southeast Asia, has also been formed with the support of UNESCO, UNDP, SEAFDEC and the host governments. □

nology be created with the main task of formulating overall science and technology policy, and conducting and supporting research and other activities in various fields. However, rather than accepting the proposal, the government will probably modify the structure of the Ministry of Industry to encompass the task of the proposed new ministry as well.

Conclusions

Science and technology in ASEAN countries needs much more planning and stimulus both from local and outside sources in order to reach a level of development comparable to, say, South Korea or Australia. For example, a mechanism is lacking whereby the results of locally supported research can be evaluated effectively. This may require the consultation of experts from outside the country, who can give critical opinions without bias. Planning of future programmes can also be more effective with such consultation.

These and other problems in research and development would require more collaboration with international bodies. Academic societies would be potential initiators of such collaboration. The proposed formation of International Chemical Society with key objectives of providing scientific aids to developing countries is interesting in this respect. Existing bodies such as Federation of Asian and Oceanian Biochemists can also provide some help. The main effort must, however, come from the governments of the ASEAN countries themselves since they are by far the largest sponsors of research and development. That international co-operation on the government level is an important factor has been realised on many occasions, including the 1975 Meeting of Directors of National Councils for Science Policy and Research in Asia and Oceania held in Kuala Lumpur. Much more effort is needed still to translate the plans into action. □

news and views

Carbohydrates recognised

from R. Colin Hughes and Nathan Sharon

THE role of complex carbohydrates in biological recognition is now recognised as being of great importance. Glycolipids and glycoproteins are widely distributed in nature, and are strategically located, at the interfaces between cells for example, where their sugar moieties serve as sensors for antibodies, hormones, toxins, interferon and infectious agents. Moreover, sugars on proteins are important in the removal of the protein from the circulation by particular tissues. Similar signals on cells seem also to be important in the movement of cells through the circulatory system, and may be involved in the cell sorting which takes place during embryogenesis. The questions now being asked, and which were discussed at a recent conference*, are how are those specific recognition determinants displayed on complex carbohydrates, whether in solution or on cells, and how do the carbohydrate sensors, after perturbation, transmit specific signals. One incentive for asking such questions is their immediate relevance to medicine, in areas such as protection against infectious disease agents, and for targeting of drugs and enzymes to sites where they would best achieve their therapeutic effects.

Because carbohydrate chains are not primary gene products, their assembly is regulated by the specificity of a large number of enzymes acting in concert. Many of the resultant structures now being elucidated show variations on a general theme. Thus, all asparagine-linked carbohydrate units contain the same Man $\alpha(1\rightarrow6)$ [Man $\alpha(1\rightarrow3)$] Man $\beta(1\rightarrow4)$ GlcNAc $\beta(1\rightarrow4)$ GlcNAc core structure. This core structure is modified by the addition of mannose chains, of sialic acid-galactose-N-acetylglucosamine chains or of both types of side chain (A. Kobata, Kobe University, Japan). In surface glycoproteins of virally transformed cells, extra branches appear to be introduced by multiple substitution of the peripheral mannose of core sequence by the sialic acid-containing

sequence, and this correlates well with malignancy (M. C. Glick, University of Pennsylvania).

Although enzymes are apt to make mistakes because their specificity is not absolute, the glycosyltransferases and glycosidases involved in carbohydrate chain assembly are turning out to be remarkably specific (R. D. Hill, Duke University; S. Kornfeld, Washington University, St Louis). In the recently discovered pathway for the biosynthesis of the asparagine-linked carbohydrate chains of glycoproteins for example, the common step involves formation of a large oligosaccharide consisting of 9 mannose, 2–3 glucose and 2 N-acetylglucosamine residues. Later on it is trimmed by specific nonlysosomal (at least four) glycosidases to a pentamannose-di-N-acetylglucosamine core structure which then may be elongated by further sugar attachment. A transferase involved in attachment of N-acetylglucosamine to the mannose- $\alpha(1\rightarrow3)$ -mannose- $\beta(1\rightarrow4)$ -sequence of the intermediate triggers trimming of two mannose residues linked to the adjacent $\alpha(1\rightarrow6)$ -linked branch. A separate transferase then can attach N-acetylglucosamine to this branch. In certain lectin-resistant cell lines, the enzyme responsible for the attachment of the first N-acetylglucosamine is missing, and glycoproteins containing only the partially trimmed pentamannose units are formed (Kornfeld; P. Stanley, Albert Einstein College of Medicine, New York). This explains the inability of the cells to bind certain lectins, enabling them to withstand lectin toxicity.

Polypeptides which are even less glycosylated are formed in cells poisoned with tunicamycin. The assembly of enveloped viruses such as vesicular stomatitis virus (VSV) grown in such cells is usually defective. This seems to be due to the instability of the nonglycosylated envelope polypeptide, its tendency to aggregate inside the cells, and its inability to be translocated to the budding site at the surface. When cells are infected with VSV (Orsay) at 30 °C instead of 38 °C, the polypeptide is more stable, and assembly proceeds almost normally (Kornfeld). These and other studies

seem to indicate that the carbohydrate in glycoproteins may stabilise polypeptide conformation, and may not be an obligatory signal for secretion.

On the other hand, the role for carbohydrate in uptake phenomena is beyond dispute. The rapid developments in this area, an outgrowth of the demonstration by G. Ashwell and A. Morell of the importance of galactose terminals for the uptake of serum glycoproteins into liver hepatocytes, are most evident in the study of lysosomal enzymes. E. F. Neufeld (NIH, Bethesda) described earlier work showing that lysosomal glycosidases all of which are glycoproteins, can be taken up readily by cultured fibroblasts, and that this uptake is abolished by periodate oxidation of the enzymes. Subsequently, the uptake was found to be affected by a variety of sugars and their derivatives, one of the most potent of which is mannose 6-phosphate (W. S. Sly, Washington University). Although this structure has not yet been reported in animal glycoproteins, G. W. Jourdain (University of Michigan) presented evidence for phosphorylated mannose containing carbohydrate chains in testicular glycoproteins which are powerful inhibitors of uptake of purified testicular β -galactosidase by fibroblasts. The notion held until very recently (see *News and Views* 209, 288; 1977) that this recognition system is the major pathway for the cycling of lysosomal enzymes from synthetic sites to the outside of the cell and their re-entry into lysosomal vesicles, was made less likely because specific antibodies or sugar inhibitors (mannose 6-phosphate) fail to deplete the cells of such enzymes by capture outside the cells. Rather, the mannose-6-phosphate is now assumed to be responsible for the segregation of lysosomal enzymes within the cell, in distinction from enzymes for secretion.

Although carbohydrate seems to be the predominant recognition marker in

*A Conference on Complex Carbohydrates in Biological Recognition was held at the Fogarty International Center, NIH, Bethesda on 17–19 July. It was organised by V. Ginsburg and N. Sharon.

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many systems, evidence was presented that some recognition processes may be dictated by a combination of both carbohydrate and protein. A case in point is the glycoprotein molecule isolated from the erythrocytes of different individuals with M or N blood group specificity. Here, specificity appears to require both sialic acid residues as well as the amino terminal serine in M active glycoprotein and leucine in the N-active molecule (E. Lisowska, Polish Academy of Sciences, Wroclaw). Other examples are the glycoprotein factors which are responsible for sexual agglutination of opposite mating types of the yeast *Hansenula wingei*. Type 21 factor, a protein containing little sugar, has a combining site which requires a determinant on the opposite Type 5-factor; the latter determinant depends on both

carbohydrate and peptide structures (C. E. Ballou, University of California, Berkeley).

Much is being learned about the attachment of biologically active peptides to cells from studies of the action of hormones and toxins, especially of the cholera toxin (R. O. Brady, NIH). In the latter case the carbohydrate recognition site on the cell surface has been identified on the ganglioside GM₁. Events subsequent to binding have been clarified in considerable detail, and include dissociation of the toxic subunit of cholera toxin from its sugar-binding subunits and penetration of the former subunit to membrane sites occupied by adenylyl cyclase. Modification of the cyclase is catalysed by a fragment of the toxic subunit, resulting in activation of the enzyme, derangement of cell metabolism and cell death. □

Two-stage multiwire proportional chambers for high flux operation?

from Derek Imrie

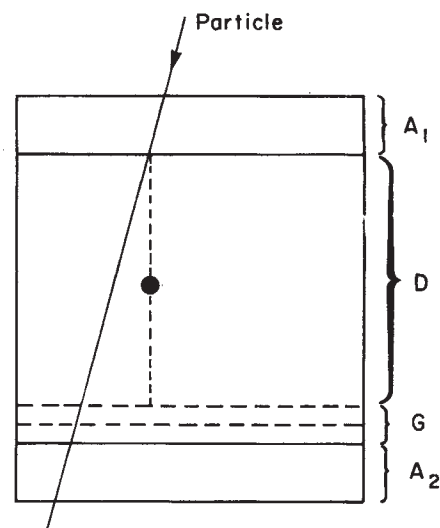
PARTICLE physicists are continually searching for ways of making their detectors operate in higher fluxes. The motivations for the quest are many: to take full advantage of the very intense beams produced by modern accelerators, to enable rarer processes to be studied, or simply to improve the statistical precision of the experimental data. The excellent time resolution and relatively short memory of the multiwire proportional chamber (MWPC) are the principal reasons for its dominance in current experiments, where large area counters having a spatial resolution of the order of 1 mm are required to operate in intense particle fluxes. However, the deterioration in performance of MWPCs at high rates has been the limiting factor in several recent experiments and an increase in the rate capability of these detectors is clearly desirable. An indication that very significant improvements may be possible is provided by a recent preprint from G. Charpak and his colleagues from CERN, CEN Saclay and the University of Warsaw (Charpak *et al.*, CERN 78-05, 1978).

The principal high flux limitation of a MWPC is the reduction in the gas gain due to the build-up of space charge in the chamber. When an electron, produced by the ionisation of the chamber gas by an incident particle, approaches an anode wire, gas multi-

plication occurs and the resulting electrons are collected by the anode, leaving a cloud of some 10^5 positive ions surrounding the wire. The ions drift slowly to the cathode where they are neutralised. In an incident flux of the order of $10^4 \text{ mm}^{-2} \text{ s}^{-1}$ the equilibrium density of positive ions in the chamber reduces the electric field in the vicinity of the anode wires, and therefore the gas gain, by an appreciable factor. Some of the reduced-amplitude anode pulses are too small to trigger the electronic circuits connected to the anode wires and, as a consequence, the chamber efficiency falls.

Unfortunately, the obvious remedy of operating the chamber at a lower gas gain (thereby producing fewer positive ions per avalanche and a lower positive ion concentration for a given incident flux) and recouping the difference by using higher gain amplifiers in the anode circuits is not feasible. Most MWPCs operate in very noisy electrical environments and the electronic thresholds are already set as low as practicable. The solution proposed by Charpak *et al.* is shown in the figure.

The chamber is divided into two parts, A₁ and A₂, separated by a drift space D, arranged so that electrons leaving A₁ arrive at A₂ after a time t_D . Region A₁ produces a gain M₁ and region A₂ a gain M₂, so that the overall gain of the system is $M = M_1 M_2$. A₂ is a conventional MWPC whose electronic threshold is adjusted to ensure that only electrons multiplied by the full gain M are detected. At the end of



Principle of the two-stage MWPC. A₁, A₂ proportional chambers; D, drift space of length d ; G, gate capturing or transmitting electrons according to controlled pulse voltage; ●, electron extracted from A₁.

the drift space, separating D and A₂, there is a grid arrangement G, that can be biased either to collect all electrons traversing the drift space or to transfer a large fraction of them to A₂. In normal MWPC gases the electron drift velocity is of the order of $4 \times 10^4 \text{ m s}^{-1}$. Therefore if D has a length of 10 mm, t_D is approximately 250 ns: long enough for electronic circuits connected to scintillation or Cerenkov counters to decide whether the incident particle is of interest or not. Only for tracks of interest would the gate be opened to allow electrons from A₁ to reach A₂. Since A₂ operates at a gain a factor of M₁ lower than normal it can be used in particle fluxes a factor of M₁ larger than those which normally produce space charge effects, providing, as is usually the case, that the particles of interest are a negligibly small fraction of the total flux traversing the chamber.

The two-stage MWPC has other incidental advantages over the conventional detector. The wire spacing of large MWPCs is usually greater than or equal to 2 mm, because of the difficulties of supporting the anode wires and ensuring stable operating conditions at smaller spacings. Obtaining the required gain in two stages reduces these difficulties very appreciably. Second, the logic delay t_D , which is an intrinsic feature of the two-stage chamber, is obtained in conventional readout systems by electronic delays, which may be difficult to equalise, sensitive to temperature or power supply variations and introduce undesirable dead time, or by cables, which are bulky, inflexible and relatively expensive.

Charpak *et al.* have tried two

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schemes for the preamplifier A_1 , but only one seems promising for particle physics applications. This is a parallel-plate proportional counter having a wire plane anode through which pass some of the electric field lines from the cathode. With this device the authors have succeeded in obtaining gains of up to 500 and a transfer efficiency into a second MWPC, across a 3.5 mm drift space, of greater than 25%. The detector worked better with a second drift space upstream of the preamplifier, but even without this addition it proved possible to operate the second chamber in a region where it detected particles whose primary ionisation had been pre-amplified, with 100% efficiency, but was insensitive to minimum ionising particles that traversed it directly. Although a gate between A_1 and A_2

was not used in these tests the authors have demonstrated the feasibility of constructing suitable gates in subsidiary experiments. The principal difficulty is to minimise pick-up of the signals applied to the gate electrodes by the A_2 anode circuits.

It is certainly too early yet for physicists to rush to their drawing boards to design experiments utilising MWPCs in fluxes one or two orders of magnitude higher than are feasible at present. A good deal of detailed experimental work and careful engineering will have to be done before the two-stage MWPC emerges as a standard piece of experimental equipment, but the preliminary results are most encouraging and future developments will be followed with great interest by the particle physics community. □

Genes, chromosomes and differentiation

by Miranda Robertson

EVEN under the broadest definition of differentiation, a significant number of contributions to the Francqui Colloquium on Differentiation* had no direct bearing on its theme. The most conspicuous of these departures was due to the presence of a critical mass of participants whose current pre-occupation is the eukaryotic split gene. The consequence was a small explosion of speculation on the basis of the recent data on eukaryotic chromosomal DNA.

The principal source of those data at the meeting was P. Chambon (University of Strasbourg), whose laboratory was the first to report that the ovalbumin gene is split by at least two intervening sequences. Further restriction mapping and electron microscopy now show that it is split by at least six (Garapin *et al.* *Cell* **14**, 629; 1978) or seven (Dugaiczky *et al.* *Nature* **274**, 328; 1978); and more refined mapping may reveal still more. In the meantime, a further complexity is added by the discovery of a short leader sequence on the mature ovalbumin mRNA (McReynolds *et al.* *Nature* **273**, 723; 1978). This sequence is not present on any of the ovalbumin genes so far cloned and must therefore be at the far end of yet another intervening sequence upstream of the 5' end of the coding region.

The immediate question raised by fragmented genes is of course how they are spliced to produce the mature messenger RNA. There is now good evidence in the case of beta globin that the splicing operates on a large precursor mRNA transcribed directly

from the chromosomal DNA (Tilghman *et al.* *Proc. natn. Acad. Sci. U.S.A.* **75**, 1309; 1978). Chambon has preliminary evidence for such a precursor for ovalbumin mRNA, in a roughly 12,000-base-pair nuclear RNA molecule which hybridises with both intervening and coding sequences on the chromosomal gene.

The intervening sequences are presumed to be snipped out of the precursor by a splicing enzyme (Knapp *et al.* *Cell*, **14**, 221; 1978) Farrell *et al.* (*Nature* **274**, 438; 1978) have just reported the discovery of an activity in yeast cells which will produce mature mRNA from a large precursor transcribed from split tRNA genes), which means there must be some recognition signal at the junction between the intervening and the coding sequence. Chambon reported at the meeting preliminary sequence data on the twelve junctions he has identified in the ovalbumin gene. His conclusions (Breathnach *et al.* *Proc. natn. Acad. Sci. U.S.A.* in the press) are substantially in agreement with those of Caterall, Brownlee *et al.* (personal communication). Since there are no complementary sequences flanking the junctions, any splicing mechanism involving the looping out of the intervening sequences as a result of base pairing between their ends seems to be ruled out. Nor at first ends seems there seem to be a unique signal sequence. Three of the intervening sequences (the first two and the last) are flanked by 4-nucleotide direct repeats: AGGT in the first, TCAG in the second, and CAGG in the last. In two of the others, there is a repeated GG and AG respectively; and one

begins and ends with a G. This apparent diversity, however, conceals an important homology: all these sequences could have been derived from a 'prototype' TCAGGT; and according to unpublished results from several sources, this homology extends to the intervening sequences of β -globin chains, immunoglobulin G λ chains and SV40. From an evolutionary point of view, it will be extremely interesting to see if it also extends to the mitochondrial genes of yeast, in the light of genetic evidence presented by P. Slonimski (CNRS, Gif-sur-Yvette) for at least two intervening sequences in the cytochrome b gene (Slonimski *et al.* *Biochemistry and Genetics of Yeast* (ed. Horecker & Stoppani) Academic Press, in the press) and a recent report (Borst *et al.* *Nature*, in the press) of restriction map and electron microscope evidence for split mitochondrial ribosomal RNA genes. Since eukaryotes so far have a monopoly on split genes, this development may put advocates of the trapped-prokaryote theory of mitochondrial origins on the defensive. However, they may be able to rescue themselves by pointing out that the genome may be less stable than we thought—some extraordinary data derived from the use of labelled cRNA probes on the polytene chromosomes of sibling *Drosophila* species (Meselson *et al.* *Proc. natn. Acad. Sci. U.S.A.*, in the press) suggest large-scale duplication and redistribution of genes at intervals during evolution (M. Meselson, Harvard University)—and the intervening sequences could have been inserted after the engulfment of the organelle. Indeed, by analogy with the directly repeated sequences found recently at either end of integrated bacterial insertion sequences, Chambon speculates that the direct repeats flanking the ovalbumin intervening sequences may be the relics of a primordial insertion event in which a circle of double-stranded DNA was broken to give complementary tails and dropped into the chromosome, where repair enzymes copied the single-stranded ends (giving the direct repeat). The origin of the inserted sequences remains beyond even the wildest speculation, but since no homology has so far been found between intervening sequences of different genes (Garapin *et al.* *op. cit.*, they do not seem to have a common source.

The absence of homology also has implications for the present function of intervening sequences, which both F. Crick (Salk Institute, La Jolla) and Chambon argue is probably regulatory in at least some cases. Absolutely nothing

*The Francqui Colloquium on Differentiation was held in Brussels on June 19–21.

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ing is yet known about the putative regulatory mechanism or mechanisms, except that they may be polymorphic: Chambon has found a phenotypically 'silent' allele of ovalbumin, in which the protein is identical but the restriction map of the gene is altered as a result of base substitutions in an intervening sequence (Garapin *et al. op. cit.*).

The pursuit of eukaryotic regulators is more advanced in the work of D. Brown (Johns Hopkins University), who refused to endorse the obsession with intervening sequences, on the grounds that the only difference they make to molecular biologists is to make it necessary to look within genes for regulatory sequences, as well as on either side. With J. Gurdon (MRC Laboratory of Molecular Biology, Cambridge), he has whittled the putative initiation sequence for the *Xenopus* 5S rRNA gene down to within 55 nucleotides of the coding sequence, by cloning repeated units and putting them into oocytes where they are accurately transcribed. He hopes to identify the beginning of the initiation sequence by removing the remaining 55 nucleotides one by one until the gene is no longer transcribed.

The cutting and splicing of nucleic acids may take place at intervals in evolution, and routinely during protein synthesis; but the general consensus at the Colloquium was that (with the sole possible exception of the immunoglobulin gene) it does not occur in the course of normal differentiation.

The issue of what does distinguish active from inactive DNA in differentiated cells was directly addressed by M. Bradbury (Portsmouth Polytechnic), who described recent extensions to the earlier discovery of a relationship between activity and the susceptibility of nucleosomes to DNase I digestion. In collaboration with V. Allfrey and others, he has now shown that susceptibility to DNase I digestion is increased by the artificial acetylation of histones; and conversely, naturally highly acetylated histones are preferentially released by DNase I digestion. A further correlate of active chromatin seems to be the presence of increased quantities of the so-called high mobility group proteins.

How these biochemical changes may determine gene expression is not known; but whatever the mechanism, it seems to be extremely stable: erythrocyte nuclei, for example, can be reactivated by transplantation into mouse L cells but they are not reprogrammed, and nor are myoblast nuclei transplanted into fibroblasts (N. Ringertz, Karolinska Institute, Stockholm).

The only known case of reprogramming is thus that of differentiated nuclei transplanted into oocytes. Ac-

Two important side issues arose at the Francqui Colloquium. One, deliberately raised by F. Crick, is the urgent need for the collation of nucleotide sequence data in some central computerised store, such as already exists for amino acid sequences.

The other issue is split-gene terminology. At present there is no consensus: some follow P. Leder and refer to intervening and coding sequences, others follow W. Gilbert and refer to introns and exons (a terminology for whose promulgation *Nature* (271, 501; 1977) must accept some responsibility), and occasionally the untranslated sequences are referred to as spacers. The arguments in favour of introns and exons are, first, that they are manageable: it is easier to refer to the intron-exon junction than to the junction between the intervening (or spacer) and coding sequences; and this means that people are likely to adopt that terminology even in the face of some other official nomenclature; it also has the advantage that there is little temptation to abbreviate. Second, they cannot be confused with anything else, whereas spacer is already applied to the non-transcribed stretches between clusters of ribosomal genes, and intervening sequence abbreviated (as it would surely be) becomes IS, the present designation of

insertion sequences in bacteria, which may or may not turn out to be a happy coincidence. The suggestion by adenovirologists (*News and Views* 274, 530; 1978), that the coding sequences could be termed 'mRNA sequences' is even more confusing: what then are we to call the sequences of mRNA.

The arguments against introns and exons fall into three categories: logical, etymological and historical. The logical argument is that, as introns and exons were coined specifically to refer to regions of DNA, they are misleading when for example, several different mRNAs are derived from one stretch of DNA, as in adenovirus, and the intron of one gene may contain an exon for another. Intervening sequences, it is argued, can apply to both. It can equally be argued, however, that any term distinguishing translated from untranslated sequences is open to the same logical objection. Etymologically, introns and exons are the illegitimate product of a Latin root and a Greek ending; and historically, they postdate the use of intervening sequence in adenovirus and in the beta-globin gene.

There is no one solution that will please everyone; but a decision must be made.

M.R.
E.L.

cording to Gurdon, the protein responsible for the reprogramming is almost certainly present in quantities too small for detection by electrophoretic methods—which were suggested by Crick. A more promising approach to this crucial question might be to clone DNA and use it to extract those proteins which bind to it and might thus be expected to have a regulatory function (Brown).

Although ultimately the control of differentiation must be nuclear, in multicellular animals nuclear differentiation is regulated by extracellular signals an important clue to whose nature may emerge from work on tumour promoters (B. Weinstein, Columbia University). The most powerful known tumour promoters are the phorbol esters, which are not carcinogenic in themselves but can induce tumour growth after a subthreshold dose of carcinogen. One action of phorbol esters is to delay differentiation (see Weinstein & Wigler, *Nature* 270, 659; 1977) and new evidence presented by Weinstein suggests that they may do so by borrowing the receptor system for a natural cellular signal: Lee and Weinstein (*Science*, in the press) find that the phorbol compound TPA inhibits

the binding of epidermal growth factor to its receptors. With the exception of hormones which (like epidermal growth factor) may affect the differentiation or proliferation of cells, the nature of extracellular signals for differentiation is largely mysterious. The only organism for which any developmental signalling molecule is known is the cellular slime mould *Dictyostelium discoideum*, which is only multicellular for part of its life cycle and owes its aggregated state to cyclic AMP. After aggregation, however, the cells require further signals to direct differentiation into either stalk or spore. J. Gross (Imperial Cancer Research Fund, London) finds that a low-molecular-weight factor ('DIF'), produced by cells plated at high density, can induce differentiation into stalk but not spore cells. For spore differentiation, the differentiating cells themselves have to be in contact.

Is it possible that major choices of developmental pathway in higher animals may be dictated by 'trivial' signals such as cell-cell contact? C. Graham (University of Oxford) has evidence in support of this idea from studies on the differentiation *in vitro* of teratocarcinoma cells. At high density,

these cells will differentiate into parietal endoderm, whereas when plated at low density they will differentiate into visceral endoderm. That applies regardless of whether the cells are in contact with other cells of the same type or with cells of a different type, and Graham believes that in such cases there is no need to postulate specific surface structures (such as epidermal growth factor receptors or 'embryonic antigens') to recognise cell-cell signals: it is not how cells make contact that matters, but whether.

However, it is clear from numerous instances of specific induction, including that described by Gross for slime moulds, that differentiation in development must be controlled in a number of different ways some of which seem

to be more specific than others.

In a final panel discussion directed at the question, "And now what?", Crick opted for tools such as two-dimensional gel electrophoresis and monoclonal antibodies as the most likely instruments for teasing out the molecules that everybody knows must exist to control differentiation but no-one can find; and J. Cairns (ICRF) speculated that cancer research directed at the study of tumour promoters may produce fundamental insights on differentiation, just as the study of tumour viruses has led to fundamental insights on eukaryotic genes. But it is debatable how far biologists can be expected to see into the future: who would have predicted three years ago that eukaryotic genes would prove to be split? □

UK geophysics—alive and well?

from Don Tarling

ARE there any lessons to be drawn from the recent UK Geophysical Assembly* at Liverpool? Certainly British geophysicists seem to be alive and consider that next year will be sufficiently productive to merit another meeting. Superficially this suggests that financial and personnel restrictions in Britain are either not too severe or that their effects are being overcome with ingenuity. Unfortunately the reality is that the effects of restrictions were pervasive. Reflection seismology reports were virtually restricted to a one-man effort to define the Hercynian front in England (Hopkins, Reading) and the conference was dominated, in quantity, by rock and palaeomagnetic studies—possibly the cheapest of geophysical investigations (other than the armchair kind).

One feature of this meeting was the way in which archaeomagnetism has reappeared fully within geophysics. Archaeomagnetism has had a chequered career in Britain, with Aitken and his colleagues at Oxford dropping the subject in preference for thermoluminescent dating when they discovered the seriousness of magnetic refraction by the kilns from which they were taking samples. British interest was renewed at Newcastle, in cooperation with the Ancient Monuments Laboratory of the Department of the Environment, using the directional properties of both fired and deposited materials. More recently, major advances have been made at both Oxford and Liverpool in deter-

mining ancient geomagnetic field intensities. These have the advantage that the original orientation of the material is not required so that pieces of pottery can be used or even adobe mud bricks (Games, Liverpool). However, it is heartening to learn that the basic principles are still being investigated—Fox *et al.* (Oxford) demonstrating that errors of as much as 20% can be introduced from the self-demagnetising effect of the shape of the samples used for the study. Despite such possible errors, the Oxford group showed clear evidence for very low values and high rates of change of the geomagnetic field intensity in Britain at the time of the Boudicean Revolt (~AD 60) that differ from observations only 750 km away in Europe, thus further supporting Creer's (Edinburgh) evidence for significant 'stationary' non-dipole field effects although his observations were based on lake sediment magnetisations on a larger scale and time-span. One significant difference between Aitken and other workers, some 15 years ago, is that all of Aitken's basic observations were published, but while Thelliers' record of directional changes in France for the last 2,000 years and du Bois' curve for changes between AD 600 and 1600 in the southwestern United States were both shown (Turner & Thompson Edinburgh; Wolfmann, Arkansas), neither of these major pioneer studies has yet been published in a form in which it can be assessed.

Studies of the magnetic properties of lake sediments and magnetic anisotropy were also presented in sufficient quantity to demonstrate that these

techniques have now become fully established. The application of magnetic studies of lake sediments enables precise correlations and dating to be established within single lakes and between different lakes. Some horizons of high magnetic susceptibility, for example, can be correlated to episodes of recorded forest fires (Rummery *et al.*, Liverpool) while changes in direction of the field can be correlated between lakes throughout Europe (Creer *et al.*, Edinburgh), although it is clear that precise studies are required to ensure that such directional changes are not, as in the case of some sediments in the Bristol Channel, entirely attributable to depositional effects (Hailwood, Southampton). Hailwood gave three quite separate papers based on the application of magnetic fabric studies to entirely different problems, one of the others being to demonstrate that sediments deposited on the edge of the Bay of Biscay and Rockall were derived from Greenland, thereby leading to an argument that the early phases of anaerobic conditions of deposition in this part of the Atlantic were more likely to be attributable to a high organic content in the rivers feeding the primitive North Atlantic ocean, rather than to stagnation in enclosed basins. Another intriguing contribution was Owen's (Birmingham) attempt to explain rotational remanent magnetisation. This magnetisation is important as most laboratories tumble specimens during alternating magnetic field demagnetisation, yet certain rocks may acquire this remanence along the direction of rotation with, as yet, no physical explanation. Owen attempted to explain how such magnetisation could be acquired by an interaction between the easy directions of magnetisation and the rotating specimen or field. While such a theory clearly has some way to go, it is vital that an explanation for this behaviour is found, not merely to prevent its adulteration of standard demagnetisation procedures but, possibly more importantly, to provide yet another parameter on the magnetic properties of rocks that might well supplement the rotation hysteresis studies described by Manson *et al.* (Newcastle).

Turning to the less magnetic sections, the work of Sowerbutts (Manchester) on interactive computer graphics for demonstration of geophysical interpretations of gravity, magnetic and seismic refraction evoked considerable interest as such techniques not merely benefit teaching but

*Abstracts of the proceedings are published in the *Geophys. J. Roy. astr. Soc.* 53, 123; 1978.

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considerably improve on the speed and therefore the feasibility of trial and error testing of real problems—an approach that is still necessary when geophysical results can often be interpreted by an infinity of possible models of which the most geologically sensible must then be sought. So far such an evaluation is still a human prerogative, although one wonders for how much longer! Mussett and Dagley (Liverpool) showed how the volcanic activity of Mull is now confined to within 3 million years some 60 million years ago. Banks and Swain (Lancaster & Leicester) raised the problem of the source of isostatic compensation, a problem of major importance since the inception of plate tectonics and therefore the potentially great depths of the levels of isostatic compensation on the continents. Studies of the gravity field in East Africa now suggest that while the depth of compensation for the plateau is unknown, the smaller structure of the Kenya dome, on which the rift valleys lie, is compensated at fairly shallow depths, less than 55 km—such results being consistent with results from North America which indicate that gravity anomalies of long wavelengths, $\sim 1,000$ km or more, have perfect isostatic compensation, but wavelengths of less than 100 km are only imperfectly compensated. Rice (Newcastle) questioned the standard gravitational segregation model for the Skaergaard intrusive and provided a convective alternative that appeared equally adept at explaining volcanic differentiation or homogenising salad oil. □

Turning points in predicted protein structures

from M. Geisow

IN recent years, considerable improvements have been made in the accuracy of protein conformation derived from amino acid sequence data alone. The widespread adoption of this approach may be judged by the number of recent reviews, including two from authors in laboratories specialising in the experimental determination of protein 3-D structure (Shulze *Angew. Chem.* **16**, 23; 1977, Sternberg & Thornton *Nature* **271**, 15; 1978).

In the most general terms, there are two empirical approaches to the estimation of protein folding from sequence information. The best known of these uses the frequencies with which particular amino acids occur in α -helix, β -structure or β -turns (180° reversals

of the polypeptide chain) in proteins of known conformation. B. Robson at the University of Manchester and P. Chou and G. Fasman at Brandeis University use these statistics to determine the likely location of the same structural features in proteins of unknown 3-D structure. The other broad approach is intrinsically kinetic and is founded upon the assumption that when proteins begin to fold, local interactions (between nearby amino acids) predominate over interactions between amino acids more distantly separated in linear sequence. Many workers have used this assumption as the basis for a predictive method and V. Lim, of the USSR Academy of Sciences, has published a set of rules which allow the estimation of the total folding of globular proteins.

Since none of these methods yet competes with crystallography in precision of structure determination, their value to biochemists is restricted to applications which only require approximate structural information. A good example of the use of the empirical approach is the independent estimation of protein secondary structure for comparison with that determined by spectroscopic methods. Regions of sequence which lack regular structure can also be estimated and these indicate likely sites for proteolytic attack.

This type of strategy has been adopted by Beely (*Biochem biophys. Res. Commun.* **76**, 1051; 1977) and Aubert *et al.* (*Arch. Biochem. Biophys.* **175**, 410; 1976) who find that sugar chains seem to be preferentially attached to glycoproteins at β -turns. Beely reports that 30 out of 31 glycosylated residues occur in sequences favouring the β -turn. He points out that although the amino acid sequence Asn (sugar)-X-Ser (or Thr) is a necessary condition for glycosylation, it is not sufficient, and his analysis points to an additional conformational requirement. Carbohydrate is present at β -turns in the known structures of human immunoglobulin heavy chain and RNase B. Small *et al.* (*Biochem. biophys. Res. Commun.* **79**, 341; 1977) find that 80% of amino acids phosphorylated by protein kinases are also located in sequences predicted to be in the β -turn conformation.

It may be that the enzymes responsible for these post-translational modifications require a particular conformation of their target amino acids, but an overriding consideration must be that such sequences are exposed at the surfaces of their protein substrates. The β -turn is certainly a surface struc-

ture, but it is only a special example of the often irregular bends and loops which comprise a substantial fraction of the surface of globular proteins. Although these features are poorly located by available treatments they are just the regions of chain most likely to be involved in intermolecular events.

An improvement in the location of these bends has been introduced recently by Rose (*Nature* **272**, 586; 1978). When the free energies of transferring each amino acid side chain from an aqueous to a non-polar environment are plotted as a function of linear sequence, turns and loops appear as local minima, between maxima which represent regions of the polypeptide chain which form the hydrophobic protein core. This is a welcome adjunct to the current empirical prediction techniques, since the algorithm described has a firm thermodynamic basis.

With the help of these methods many potential sites of post-translational modification, such as limited proteolysis or adenylation, can be explored using existing amino acid sequence information. However, the correlation of acceptor sequences with predicted structural features should be made with some caution. One must first be satisfied that the modification of interest occurs after the full tertiary structures of the molecules are acquired. For example, Lingappa *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 2338; 1978) show that the glycosylation of α -lactalbumin only occurs during its synthesis on the ribosome. Pless *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74**, 134; 1977) find that a variety of glycoproteins can only serve as carbohydrate acceptors if they are supplied to the enzyme system in their denatured state. The lack of structure during this particular modification obviously weakens the argument for a structural basis of the observed specificity. With this proviso, the methods discussed here may well prove powerful in locating or confirming potential sites of biochemical modification of globular proteins. □

100 years ago

It is stated in the French papers that Mr. Edison is to have no reward whatever at the Paris Exhibition for his phonograph. The reasons alleged for this apparent denial of justice are somewhat amusing. The jury of the class of instruments of precision declared that the phonograph could not be considered as at all an instrument of precision, but merely a toy; consequently they sent it to the class of telegraphy to be rewarded. But the telegraphists replied that it was of no use whatever in telegraphy, and refused to examine it. The consequence is that the most wonderful invention, probably, in the Exhibition, will be passed by unmentioned and unrewarded.

From *Nature* 18, 15 August, 424; 1878.

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Plant tissue culture in China—major change ahead?

from Norman Sunderland and Edward C. Cocking

PLANT tissue culture techniques are now recognised as an important tool in programmes of crop and plant improvement. In the People's Republic of China emphasis has been given in the past mainly to anther culture and its role in haploid breeding. About 200 teams comprising over 2,000 workers have been established, based in laboratories at the commune, county and prefectural levels and coordinated by scientists in the Universities and Institutes of Academia Sinica. This massive concerted effort, designed for the rapid production of higher yielding and more disease resistant varieties, has already resulted in improved lines of rice, wheat and tobacco, many hectares of which are now under cultivation. Success with maize and brassicas may be expected to follow soon. With the new drive into science, however, inaugurated earlier this year by Chairman Hua, the use of culture techniques is to be extended to provide other approaches to crop improvement. Various aspects of plant genetic manipulation are among the topics designated for special attention.

In May and June we had the opportunity of visiting China under the auspices of Academia Sinica and the Royal Society to participate in a Symposium on Plant Tissue Culture organised by Academia Sinica and the Australian Government. Five scientists from Australia and ten from eight other countries attended the Symposium in Peking. The Symposium was followed by an extensive tour of

Universities, Research Institutes and communes in Peking and in other parts of the country.

The changing attitude of Chinese leaders to fundamental science was outlined by Vice Premier, Keng Piao, in a televised interview held in Peking's Hall of the People. Very encouragingly he stressed the importance of basic studies on plant cell cultures in his country's desire to apply this new technology to crop improvement. China wanted exchange of research workers and also close collaboration between the hundreds of Chinese scientists who will be involved in the new ventures and the scientists of other countries.

Mutual cooperation and friendship between plant scientists were the keynotes of a reception given by Academia Sinica in the evening preceding the Symposium. Two films were shown describing the development of new varieties of tobacco and rice by anther culture. Both were in colour and of high standard: they expressed simply and clearly the genetic basis of the method and showed the various steps being carried out on the communes. These films admirably demonstrated one of the ways in which scientific principles and methods are being brought to the people in China. Foreign guests received samples of the first cigarettes to be manufactured from a new tobacco variety developed in Shantung Province from haploids.

The Symposium, which was the first International Biological Symposium

held since the Revolution, lasted for five days and followed traditional Western lines. The first day was devoted to 30-minute review papers given in English by Chinese and foreign participants, without discussion. Morning sessions thereafter followed a similar format, but afternoons were set aside for formal discussion sessions organised by the Australian group. Informal evening discussions were also arranged between individual foreign participants and groups of young Chinese working in similar fields. Many of these younger Chinese researchers had never previously had the opportunity of meeting foreign scientists. Foreign guests had the opportunity of reaching a wider audience in a series of public lectures attended by scientists, technicians and teachers. The Symposium was widely covered by the press and synopses of lectures given by foreign guests were printed in the local newspapers.

Among the 47 papers delivered at the Symposium, 19 related to anther culture principally of rice, but also of wheat, maize, tobacco and rubber. The Chinese attitude is clearly to adopt a simple workable protocol which is then carried out without deviation by hundreds of hands. There was some indication of competition between groups largely in respect of the use of culture media: some favour synthetic media such as the newly developed N6 medium whereas others favour media supplemented with potato extract. There seems to be no desire to identify the active principles in the potato.

In cereals a high proportion of plants derived from tissue culture are albino, and therefore useless. There has been much discussion over whether this undesirable phenomenon has a genetic or physiological basis. The Chinese seem to favour the physiological view. They have shown that the ratio of green to albino plants can be influenced by temperature, and biochemical and ultrastructural studies have revealed specific protein and RNA deficiencies in the albino plastids.

Production of cereals from tissue culture is predominantly through induction of callus tissue; some groups however are attempting to use the alternative route of embryo culture.

Ten papers dealt with protoplasts and many of these described Chinese work on protoplast isolation, culture and fusion. As yet no somatic hybrids have been produced in China, but there

Professor Loo Shih-Wei and his welcoming group at the Laboratory of Cell Physiology, Shanghai Institute of Plant Physiology, Academia Sinica. The poster at the back gives a welcoming message in Chinese.



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is already extensive work on isolation including the use of cellulase produced in the Institute of Plant Physiology in Shanghai. K. J. Scott (University of Brisbane) reported success in the division of barley leaf protoplasts using a special rapid method of isolation and sequential media incubations; and it was exciting to see in a visit to the laboratory of Li Hsiang-Hui in the Institute of Genetics, Peking, that he was able to obtain division of wheat leaf protoplasts after isolation by means of Chinese cellulase, and incubation in a relatively simple culture medium.

The post-symposium tour took the party to Shanghai, Hangchow, Kweilin, Nanning and Kwangchow. Visits of scientific interest were interspersed with tours of factories, communes, botanical and medicinal herb gardens.

In comparison with the West conditions in the universities left much to be desired, and invariably deficiencies were blamed on the iniquities of the Gang of Four. To fulfil projects of somatic hybridisation and genetic engineering better trained students are required.

The tour also gave the party first-hand experience of haploid breeding. Particular mention may be given to the work on wheat seen in the Red Star and Tung Pei Wang communes near Peking, on rice at the Chung Po County Laboratories and Chin Chow, and on maize in the Corn Research Institute and the Kwangsi Agricultural University, Nanning. Development of future programmes of transferring nitrogen fixing genes into cereals and of somatic hybridisation will be watched with interest. □

rather than the reed (*Phragmites australis*). Physical damage from boat traffic rather than eutrophication is a likely cause of this change.

To ornithologists the Broads are closely associated with the bittern (*Botaurus stellaris*), a reedswamp bird which began recolonisation of the area in 1911 after 50 years during which only three records of breeding exist. Day and Wilson (*Brit. Birds* 71, 285; 1978) have collated information on the expansion of this species in Britain since that time, but they also have bad news for the Broads. By 1954, 60 pairs were nesting in Norfolk (about 75% of the British breeding population of the time) but this was reduced to 27 pairs by 1970 and to 10 pairs in 1976 (about 20% of the birds breeding in Britain).

The decline has not been seen in other British counties. The bittern population of Suffolk has risen by 50% over the same period. It is therefore unlikely that any non-local factor, such as hard winters, has been involved. Day and Wilson also produce data concerning the density of breeding herons (*Ardea cinerea*) in the Broads. They declined abruptly following a hard winter in 1962/63 as did the bitterns but they have subsequently recovered to their former level. The bitterns show some recovery, but the general decline in numbers since 1954 has continued.

The explanation must lie in a deleterious local factor such as pollution, habitat destruction or disturbance. The answer may well lie in the simplified food chains resulting from eutrophication and increased turbidity. The bittern, unlike the heron, seems unable to cope with such habitat changes. The Broads thus seem to be another classic of conflicting interests—wildlife conservation and recreation being the two main opponents. The conventional British compromise of partition is an incomplete solution to this kind of problem where the Broads are interconnected waterways and where many of the best wildlife areas, such as Hickling, Horsey and Heigham Sound are also the most popular sites for sailing. Where entire river catchments form the basic ecological unit for management, sub-division of the waterways among the conflicting interests alone will not solve the problem. □

The Norfolk Broads under pressure

from Peter D. Moore

THE conservation of fresh water habitats and their associated swamp and fen vegetation is rendered difficult both by the natural process of succession and by the vulnerability of such ecosystems to human misuse. The Norfolk Broads in eastern England provide a good example of how these two forces can interact to produce an ecological headache.

The open water areas of the Broads were themselves produced by Mediaeval peat-cutting and so can be regarded as a man-made environment. The value of this series of lakes and interconnecting rivers for recreation has long been recognised, although its exploitation is fast increasing; the number of power-boat licences issued has risen from 1,250 in 1947 to 9,247 in 1976. Boating interests are naturally concerned to maintain open, navigable waters, but the natural processes of secondary succession have tended to reduce the open water areas as reed-swamp encroaches. Ellis (*The Broads*, Collins London, 1965) concluded that the amount of open water in the Broads had declined from 1,200 ha in 1880 to 700 ha in the 1950s. During the following decade there was an increase in open water as a result of the grazing activity of the introduced coypu. The coypu population is now controlled, but no further encroachment by vegetation seems to have taken place.

The current state of the aquatic flora and fauna of the Broads has been

reviewed by George (*Trans. Norfolk Norwich Nat. Soc* 24, 41; 1977). He describes experiments which are in progress using 20-m diameter Lund tubes which isolate a body of water and sediment from its surroundings. Preliminary results suggest that some of the characteristic Broads water plant species which have recently declined, such as *Potamogeton pectinatus* and *Najas marina*, germinate widely but survive only in the tubes. It is not yet clear whether some chemical factor, or turbulence created by boats is involved in their inability to survive.

Mud sedimentation rates have increased dramatically in the past 30 years and this can probably be ascribed to higher phytoplankton productivity resulting from eutrophication. Jackson (*Trans. Norf. Nor. Nat. Soc.* 24, 137; 1978) has conducted a survey of the macrophyte flora of the Broads and has concluded that there has been a serious decline in the diversity and abundance of water plants in the area. Much of the eutrophication of the Broads results from sewage treatment plants, but the contribution of agricultural run off and from boats has been considerable. George describes fish kills which have been associated with toxin production by phytoplankton components and a decline in benthic invertebrate diversity. Chironomid midge larvae and tubificid worms have replaced a previously rich fauna.

George also points out that the marginal reedswamp, which itself once threatened the survival of open water in the Broads, is now declining. Tussock fen often borders the waters

Correction

In the article 'Monkeys prefer kin' (*News and Views* 274, 311; 1978) Wu and Sackett's work was carried out with the pig-tailed macaque (*Macacca nemestrina*) and not the rhesus monkey as stated.

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review article

Cellular transformation and the 'morphologic phenotype' of transformed cells

Ira Pastan & Mark Willingham*

Expression of the product of the transforming gene (src) of RNA tumour viruses promotes growth and usually alters the adhesion, appearance and surface properties of cultured fibroblasts. The latter group of properties termed the 'morphologic phenotype' of transformed cells is largely due to diminished cell-to-substratum adhesion. The role of cyclic AMP, cell surface protein (CSP), and other factors in producing the 'morphologic phenotype' are discussed. The effects of src expression bear a striking resemblance to the action of peptide hormones such as insulin on appropriate target cells.

THE transformation of fibroblasts, particularly by RNA and DNA tumour viruses, has been extensively studied with a view to understanding the biochemical events responsible for the abnormal properties of cancer cells. Transformed cells, as well as having abnormal growth, are usually recognisable by their rounded shape, disordered alignment, low adhesion to substratum, enhanced agglutinability by plant lectins and the presence of microvilli or other irregularities on the cell surface¹. There is considerable evidence that the latter group of properties, which have been referred to as the 'morphologic phenotype' of transformation, is secondary to diminished adhesion to substratum. Two biologically important molecules have been implicated in controlling expression of the transformed phenotype—cyclic AMP (a molecule found inside cells) and a major cell surface protein commonly called CSP (cell surface protein)², fibronectin³ or LETS⁴ (large external transformation-sensitive protein). We discuss here the evidence for the biochemical basis of the morphologic phenotype of transformed cells, and speculate on the nature of the biochemical events that produce the changes in cell appearance and growth control.

Cyclic AMP

In 1969, treatment of cells with dibutyryl cyclic AMP (Bt₂ cyclic AMP), an analogue of cyclic AMP, was found to change the appearance of L929 cells, Chinese hamster ovary (CHO) cells, and some other transformed cells so as to induce the flattened appearance characteristic of normal fibroblastic cells⁵⁻⁷. Accompanying this change was an increase in adhesion to substratum⁸, decreased agglutinability by plant lectins⁹⁻¹¹, a decrease in the number of surface microvilli¹² and a decrease in growth rate^{9,13-15}. But contact inhibition of growth was not restored¹⁵. One important point that is often overlooked is that not all transformed cells respond to cyclic AMP treatment. The reasons for the variation are not yet clear, but one probable determining factor is the embryonic origin of the cell. The terms 'fibroblast' or 'fibroblastic cells' are used rather indiscriminately to describe a flattened cell that looks something like a cultured mature skin fibroblast. However, flattened cells propagated in tissue culture are often derived from nonfibroblastic cells.

One very good guide to whether a transformed cell will respond to cyclic AMP treatment is the response of the parental untransformed cell. For example, when normal Swiss 3T3 cells are treated with Bt₂cAMP, they become even more flattened and elongated than the untreated cells^{15,16}. Transformed Swiss 3T3 cells are very responsive to treatment with cyclic AMP analogues. On the other hand, a clone of 'epithelial' normal rat kidney (NRK) cells is unresponsive to cyclic AMP, and so are its transformed derivatives (see below).

In some normal cells that are affected by cyclic AMP treatment, crowding and contact inhibition of growth are accompanied by a natural increase in cyclic AMP levels. Such an elevation in cyclic AMP levels at or near confluence has been observed with Swiss 3T3 cells¹⁷, NRK cells^{18,19} and one but not other clones of BALB/c 3T3 cells (refs 20 and 21 and I.P., unpublished data). Although serum starvation may account for the rise in cyclic AMP in some cells (reviewed in ref. 22), cyclic AMP also rises in the absence of serum starvation (ref. 22 and below). Transformed derivatives of Swiss 3T3 cells and NRK cells cease to show raised cyclic AMP levels at confluency. In the case of murine sarcoma virus-transformed cells this is associated with a decrease in the ability of adenylate cyclase to be activated by prostaglandin E₁ (ref. 23 and I.P., unpublished data). Cyclic AMP levels have not been observed to rise in confluent chick embryo fibroblasts¹⁹ and some lines of BALB 3T3 cells (refs 20 and 21 and I.P., unpublished data).

An example of cells that vary in their response to cyclic AMP treatment or in their ability to alter their cyclic AMP levels, is provided by recent studies on NRK cells, a line of cultured rat kidney fibroblasts. Early reports stated that cyclic AMP levels of NRK cells are raised at confluency, and this no longer happens after transformation by murine sarcoma virus (MSV)¹⁸. It was also found that Kirsten sarcoma virus (KISV) transformants (KNRK) responded to cyclic AMP treatment by becoming flattened and elongated²⁴. Later investigators, however, failed to detect a change either in cyclic AMP levels in MSV transformed NRK cells²⁵ or in morphology when NRK cells were treated with 8-BrcAMP²⁶. However, De Larco and Todaro²⁷ recently reported that the original NRK culture contained both an elongated fibroblastic cell type and a cell type (VB4) which is polygonal or epitheloid in appearance. We thought it important to compare the metabolism of cyclic AMP in these two cell types and their transformed derivatives. We

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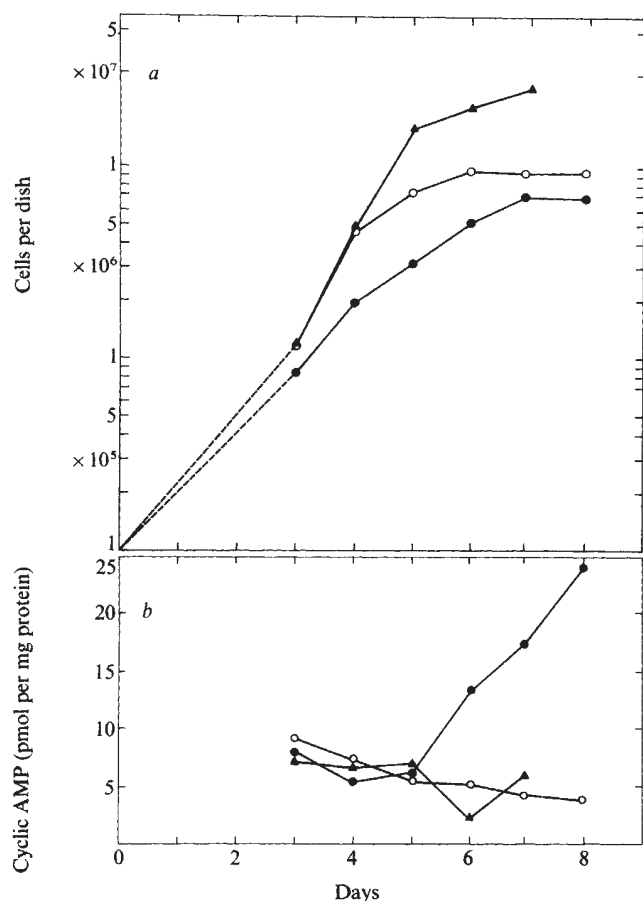


Fig. 1 Cell growth (a) and cyclic AMP (b) levels in fibroblastic (NRK-2) (●) and epitheloid (NRK-VB4) (○) NRK cells and a MSV transformant of the fibroblastic cell line (▲).

have confirmed that confluency is accompanied by raised cyclic AMP in the fibroblastic cell type (NRK-2) (Fig. 1), and that this is not due to serum starvation. Further, the rise at confluency was lost on MSV transformation (Fig. 1). Both NRK-2 and an MSV-transformed derivative responded to cyclic AMP treatment. They grow more slowly in the presence of 1 mM 8-BrcAMP (Table 1) and this growth inhibition is accompanied by a flattening of cell shape (Fig. 2, and data not shown). In contrast, cyclic AMP levels in the untransformed epitheloid clone (VB4) are not elevated at confluency (Fig. 1) and its appearance (Fig. 2) and growth rate (Table 1) are unaffected by cyclic AMP treatment.

For cells to respond morphologically to cyclic AMP analogues they must be attached to the substratum and have a competent cyclic AMP-dependent protein kinase system. Many clones of MSV (Moloney)-transformed NRK-2 cells are rounded and have very poor adhesion to substratum (I.P. and M.W., unpublished). Cyclic AMP treatment has little effect on the round shape of these cells, although it slows their growth. Further, transformed cells maintained in suspension culture will grow more slowly in the presence of cyclic AMP analogues, but retain their rounded shape^{28,29}.

Cyclic AMP mutants

That cyclic AMP has an important role in controlling the shape of Swiss 3T3 cells has been further confirmed by the isolation of a mutant or variant of Swiss 3T3 cells in which a rapid fall in cyclic AMP can be induced by a shift in temperature³⁰. This fall in cyclic AMP is rapidly followed by decreased adhesion to substratum, rounding of cell shape, and the appearance of microvilli and enhanced agglutinability^{11,30}. This entire response can be blocked by prior treatment of the cells with

Bt₂cAMP or prostaglandin E₁, an activator of adenylate cyclase.

We have recently isolated a CHO cell line with a mutant cyclic AMP-dependent protein kinase (D. Evain, M. G. Gottesman, I. P. and W. B. Anderson, in preparation). Treatment with 8-BrcAMP has only minimal effects on the shape or growth rate of these cells although it has striking effects on the shape and growth of normal CHO cells¹⁰. This result demonstrates the importance of the cyclic AMP-dependent protein kinase system in the response of CHO cells to cyclic AMP. Earlier experiments showed that cyclic AMP treatment inhibits the growth of S49 lymphoma cells, but not mutant cells defective in cyclic AMP-dependent protein kinase³¹. Unlike CHO cells, however, S49 cells grow only in suspension, do not attach to tissue culture dishes, and do not show a morphologic response to Bt₂cAMP.

In animal cells cyclic AMP is thought to act by promoting the phosphorylation of key proteins and thereby altering their activity. Fibroblasts, like other cells, contain many phosphoproteins³². Cyclic AMP-dependent phosphorylation of some of these proteins has been demonstrated in cell-free extracts³². The most likely proteins to affect cell shape would be those associated with the plasma membrane or with cytoskeletal elements. Two such phosphoproteins have been identified. One is the actin binding protein, filamin. The other is a group of high molecular weight proteins that associate with microtubules during polymerisation. Both filamin and the microtubule-associated proteins (MAP) have been found associated with purified plasma membranes³³. Filamin is associated with bundles of microfilaments³⁴, whereas some MAPs are associated with microtubules³⁵.

It is clear that cyclic AMP treatment has striking effects on the behavior and appearance of some but not all cultured 'fibroblastic' cells, and that cyclic AMP levels are reduced and adenylate cyclase activity altered in some transformed cell lines. What is not yet clearly established is whether these changes in cyclic AMP levels normally regulate the behaviour of untransformed cultured fibroblasts, or if changes in cyclic AMP levels are responsible for the abnormal behaviour of transformed cells derived from cyclic AMP-responsive normal cells. The isolation of normal and transformed cells with mutations in the various genes of cyclic AMP metabolism should help answer this question.

Cell Surface Protein (CSP)

Many normal cultured cell lines make large amounts of a high molecular weight protein that is found attached to the cell

Fig. 2 Treatment of fibroblastic (a and b) and epitheloid (c and d) NRK cells with 8-BrcAMP for 48 h. a, c, Without 8-BrcAMP; b, d, with 1 mM 8-BrcAMP.

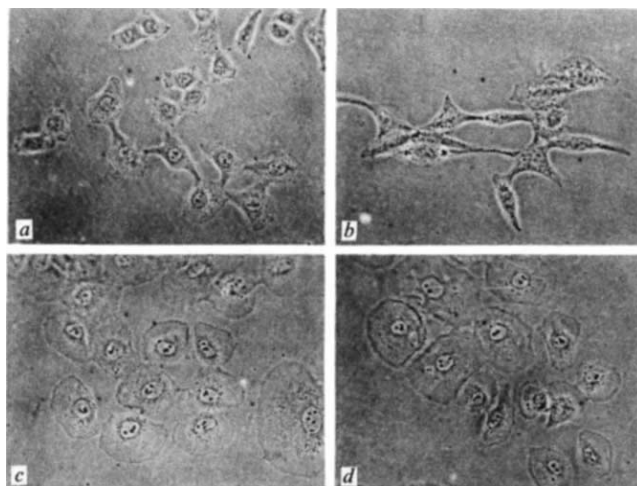


Table 1 Effect of 8-BrcAMP treatment on the growth rate of a fibroblastic (NRK-2) and epitheloid (VB4) clone of NRK cells and their MSV transformants

	Generation time (h)	
	Control	8-BrcAMP (1 mM)
NRK	20	34
NRK (MSV)	16	30
VB4	20	21
VB4 (MSV)	16	16

surface in an irregular meshwork. A decrease in CSP is seen in many types of transformed cell. In Rous sarcoma virus (RSV)-transformed chick embryo cells in which detailed studies have been performed, the drop is due primarily to a decrease in its rate of synthesis³⁶ and is accompanied by a decrease in the content of translatable mRNA for CSP³⁷.

One striking feature of CSP is its extreme susceptibility to proteolysis by trypsin. Since trypsin is commonly used to detach cells from culture dishes, it seemed possible that CSP was an adhesive protein. To evaluate this hypothesis, a method was devised to purify CSP. At neutral pH, CSP exists as insoluble aggregates on the cell surface; but if small amounts of urea are added to the culture medium it is selectively released into the medium and rapidly forms large aggregates. Yamada *et al.* found that CSP could be solubilised and further purified at pH 11 without being denatured³⁸.

The effect of highly purified CSP on cell behaviour was tested by adding it to cells in culture. In all transformed cells tested, CSP prepared from CEF was found to increase cell adhesion to substratum, and produce cell elongation³⁸; increased cell flattening was also commonly observed. However, the magnitude of the response varied. With many cell types CSP also restored the highly aligned growth pattern noted with normal fibroblastic cells³⁸. Associated with the change in cell shape was a decrease in surface microvilli and ruffles³⁹. Despite the striking morphological and adhesive effects of CSP treatment, no metabolic effects were noted. CSP treatment had no effect on growth rate, saturation density, or the increased hexose and amino acid transport observed in transformed cells^{38,40}. CSP also had no effect on cyclic AMP levels, and conversely, short term cyclic AMP treatment had no effect on CSP levels³⁸. Further, some cells that morphologically respond to CSP such as RSV-CEF, respond minimally to cyclic AMP³⁸. Clearly the two molecules act by different mechanisms.

Cell shape and loss of growth control

The observation that CSP treatment did not restore growth control, but had dramatic effects on cell shape, was not entirely unexpected, since thrombin had been showed to stimulate the growth of normal CEF without affecting CSP levels⁴¹. Further, other kinds of experiment had shown that CEF and other normal cells assume a rounded shape when placed on a type of plastic culture dish to which cells adhere poorly; these rounded cells still display normal growth control⁴².

Mutant cells with the transformed morphologic phenotype

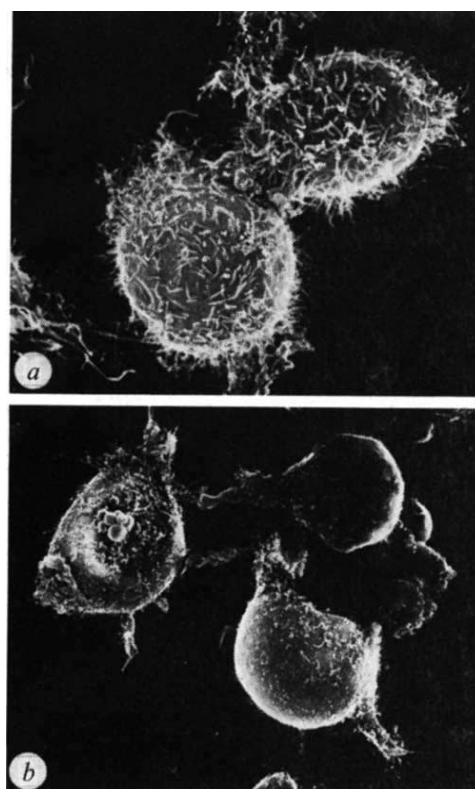
To help understand the relationship between cell shape and loss of growth control, we isolated mutant BALB 3T3 cells that were rounded and had decreased adhesion to substratum. One of these, AD6, has been studied in detail^{43,44}. The mutant cell is unable to acetylate glucosamine-6-phosphate to form *N*-acetyl glucosamine-6-phosphate⁴⁵. This defect leads to the formation of glycoproteins, proteoglycans and glycolipids with a decreased carbohydrate content. Because of this defect, adhesion to substratum is diminished. Because of diminished adhesion the cells are rounded, have more microvilli, and are readily agglutinated by plant lectins. Yet, they have a normal growth rate, do not metabolise glucose at a higher rate, and do not form tumours in animals⁴⁴. These cells have the

'morphologic phenotype' of transformed cells (Table 1), yet are not 'malignantly transformed'. It seems likely that because the surface glycoproteins that participate in adhesion to substratum are poorly glycosylated, they fail to bind to the substratum properly. Perhaps, in addition, the internal connections between the intracellular submembraneous system of microfilaments and membrane glycoproteins are affected so that adhesions to substratum are destabilised.

Agglutinability and microvilli

The enhanced agglutinability of cancer cells was first noted in studies of lymphoma cells⁴⁶. Later, a similar difference was noted when normal and transformed fibroblastic cells were compared^{47,48}. Agglutination depends on the fact that lectins are multivalent and can form bridges between cells. The enhanced agglutinability of transformed cells is not due to increased numbers of concanavalin A receptors (reviewed in ref. 12). The observation that cyclic AMP-treated transformed cells had decreased numbers of microvilli and decreased agglutinability by lectins suggested that microvilli might provide an explanation for the increased agglutinability of transformed cells¹² because they provide large surfaces which are readily cross-linked (or glued together) by lectins. Electron microscopic observations on agglutinated cells showed that almost all the cellular interactions occur at the surface of microvilli¹². In support of this proposal was the fact that agglutinability was

Fig. 3 Scanning electron microscope appearance of fibroblastic cells with high and low agglutinability fixed in suspension. L929 cells (a) (high agglutinability) and Swiss 3T3-4 cells (b) (low agglutinability) were removed from their substrate by treatment with 0.1 mM EDTA at 23 °C (5 min). They were fixed in suspension (M. C. W. and S. S. Yamada, unpublished) with 1% glutaraldehyde and slowly washed over a Millipore filter without allowing them to settle on the filter. They were then placed over a polylysine-coated coverslip and allowed to settle and attach. The coverslip was then post-fixed in 2% glutaraldehyde, 1.5% OSO_4 , and dehydrated in ethanol following by critical point drying and platinum rotary shadowing. The cells were viewed using a Hitachi HU-12A electron microscope with an HSE-2 scanning attachment. (a, $\times 1500$; b, $\times 750$).



increased in mitotic cells and in trypsinised cells. Microvilli and other cell surface irregularities are markedly increased in both these conditions. Another proposal to explain increased agglutinability was that the fluidity of the membranes of transformed cells is increased, leading to more rapid formation of lectin aggregates on the cell surface, and that these aggregates promote agglutination⁴⁹. A difficulty with this proposal is that visible aggregates are not observed until 30–60 min after lectin addition, whereas enhanced agglutinability is evident after 5 min. Another is that recent studies show that transformed cells do not have increased membrane fluidity^{50,51}.

When cells in suspension are observed by both dark field and scanning electron microscopy, fibroblastic cells with low agglutinability have fewer and shorter surface projections than their highly agglutinable transformed counterparts (unpublished observations). A typical example of cells fixed in suspension immediately after removal from the substratum with EDTA is shown in Fig. 3. Fig. 3(a) demonstrates the numerous long microvilli characteristic of L929 cells, a highly agglutinable transformed cell line. Fig. 3(b) shows the surfaces of some Swiss 3T3 cells, a cell line of low agglutinability, with fewer and shorter surface projections.

Although a relationship between microvilli and agglutinability is commonly observed, exceptions have been noted when suspended cells were examined by scanning electron microscopy^{52,53}. The reasons for these differences are not yet clear. Factors influencing the preservation of microvilli and other surface structures when cells are fixed in suspension and subjected to manipulation and critical point drying probably need to be further examined. Although the most common artefacts are loss of microvilli, they also can form rapidly. These artefacts can be evaluated by monitoring living cells by dark field microscopy to see if gross changes in microvilli occur during agglutination assays and fixation for scanning electron microscopy. Further, many cell lines show heterogeneity in their surfaces and a profile of surface morphology in a large number of cells is necessary to determine the average state during a mass phenomenon such as agglutination. Variations in the surfaces of normal 3T3 cells are evident in Fig. 3.

Cytoskeleton and cell shape

With the preparation of antibodies to actin, myosin, tropomyosin and filamin, it has been possible to visualise the structures containing these molecules by fluorescence microscopy (reviewed in 42). The structure observed with antibodies to actin, myosin and filamin appear similar to the stress fibres previously observed by phase contrast microscopy⁵⁴. The organisation of these structures is disrupted in transformed cells. It is also disrupted in rounded cells produced by culturing cells on a low adhesive substratum, in mitotic cells and in the adhesion-defective mutant AD6⁴². Further, the assembly of bundles of microfilaments is promoted by conditions that enhance adhesion. The latter include CSP addition, cyclic AMP treatment and correction of the biochemical defect in AD6 by feeding *N*-acetyl glucosamine⁴². For these reasons it seems likely that it is the defect in adhesion which is responsible for the altered morphology observed in transformation. Furthermore, it seems unlikely that a defect in assembly of the microfilamentous system is a primary cause of the altered growth of transformed cells⁴².

Src gene product, cell growth and transformed phenotype

Rapid changes in cell behaviour due to expression of a transforming gene are most readily studied in cells infected with viruses containing temperature-sensitive mutations in the transforming gene (*src*). To date, most such studies have employed mutants of Rous sarcoma virus (reviewed in ref. 55). Cells infected with these mutant viruses will not grow in agar at 41 °C and have a normal appearance when propagated in tissue culture at 41 °C. However, cells transformed with such mutant

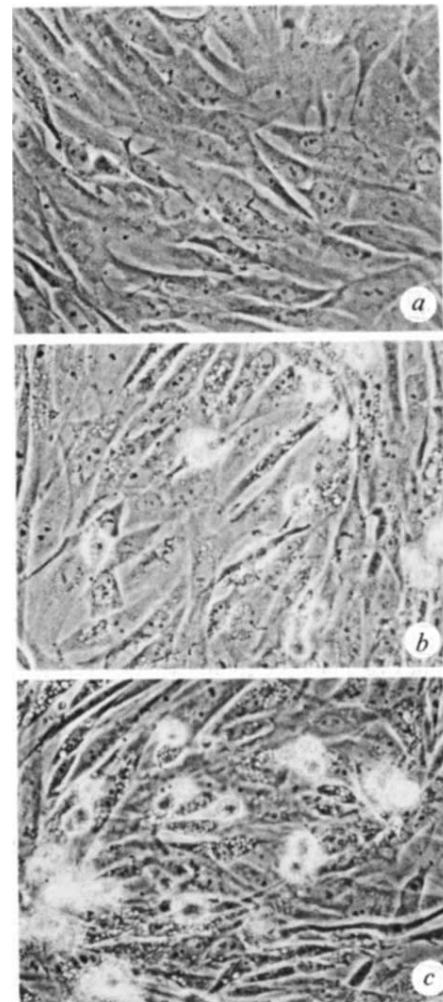


Fig. 4 Rounding and vacuolisation of CEF transformed by RSVts68 before (a), and at 1 h (b) and at 3 h (c) after shift from 41.5 to 36 °C.

viruses undergo rapid morphological and metabolic changes when placed at a permissive temperature. Bader⁵⁶ has reported a mutant of the Bryan high-titre strain of RSV in which the cells change shape and accumulate intracellular vacuoles within 10 min after a shift from 41 °C (non-permissive temperature) to 36 °C (permissive). This change in shape is evident in enucleated cells indicating that nuclear events are not required for this response⁵⁷. CEF infected with RSV-SR-ts68 also undergo a rapid change in shape when subjected to the permissive temperature. Within one hour, many of the cells have become rounded (Fig. 4).

Accompanying changes in shape in cells infected with mutant viruses, there is a drop in adenylate cyclase activity probably reflecting alteration in the cell membrane^{58–60}. Disagreement exists as to whether or not cyclic AMP levels fall in RSV-infected CEF^{60,62}. Apparently, cyclic AMP levels vary greatly in cultures of untransformed CEF. When relatively high cyclic AMP levels have been observed in normal CEF, they have been observed to fall on RSV transformation. However, when initial cyclic AMP levels are low, no drop has been detected^{60,62}. Furthermore, RSV-transformed NRK-2 cells cease to show raised cyclic AMP levels at confluency (unpublished data).

When cells infected with RSV *src*^{ts} mutants are placed at the permissive temperature, glucose transport begins to increase within a few hours and continues to do so for 6–12 h^{63,64}. The time course of these events is consistent with an action of the

transforming gene *src* beginning within one hour of the change in temperature, suggesting a direct action of *src* on the glucose uptake mechanism. Recent evidence, however, has suggested that the effect of *src* on glucose uptake may be mediated by nuclear functions⁶⁵.

RSV-transformed CEF synthesise less of two major cell proteins. One is CSP³⁶; another is collagen. Since transformed cells contain less mRNA for these two proteins³⁷, it seems likely that *src* is exerting an effect on the cell nucleus that alters the synthesis or processing of the mRNA for CSP and collagen. Decreased CSP and collagen synthesis is not specific for RSV-infected CEF. Hamster embryo cells infected with the Harvey strain of MSV have a decreased rate of LETS synthesis (R. O. Hynes, personal communication), and rat embryo fibroblasts transformed with MSV (Kirsten) strain have a decreased rate of collagen synthesis^{67,68}. A summary of some of the sites affected by *src* and their relationship to the 'morphologic phenotype' of transformed cells is shown in Fig. 5.

Src gene product and peptide hormones

Recent evidence indicates that the product of the *src* gene of the Schmidt-Ruppin strain of RSV is a polypeptide with a subunit molecular weight of 56,000 to 60,000⁶⁹⁻⁷¹. A very unusual feature of this peptide is its short half life which is about 20-30 min⁶⁹.

The changes initiated on expression of *src* closely resemble those that occur when quiescent cells are stimulated to grow by the addition of the appropriate peptide hormone. Many peptide hormones have been found to promote the growth of appropriate test cells, but for simplicity we discuss insulin as a prototype of growth-promoting substances.

Insulin binds to specific receptors on the cell surface^{72,73}. The half life of insulin once bound to the surface of cells is very short; certainly less than 30 min⁷³. Cell-bound insulin rapidly produces an increase in glucose metabolism, as well as a host of other metabolic changes⁷⁴. The increase in glucose transport is not blocked by cycloheximide⁷⁴. In fibroblastic cells, insulin has been observed to alter the membrane and promote the formation of microvilli⁷⁵. Insulin also has rapid effects on adenylate cyclase activity, causing its activity to fall²⁹. In pre-adipocytes, a cell line derived from 3T3 cells, insulin specifically alters the synthesis of proteins involved in the metabolism of lipids^{76,77}. Insulin is also a growth factor for many cultured cells⁷⁸.

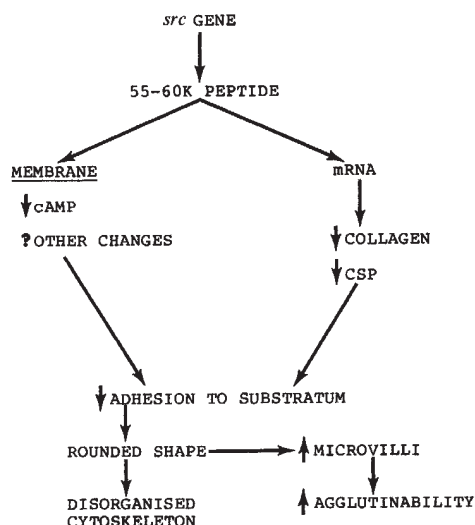
Now it is clear that the *src* gene product is not insulin. Yet, both molecules have many properties in common. Both are

peptides and have short half lives and rapid effects on glucose metabolism and other membrane-related phenomena including adenylate cyclase activity and the formation of microvilli. Both have selective effects on protein synthesis. Insulin promotes the synthesis of specific proteins, whereas *src* has been found to inhibit synthesis of some cell proteins and, by altering glucose metabolism, promote the synthesis of others⁷⁹.

One site or many sites of action

Investigators studying peptide hormone action have long been interested in whether hormones promote cell growth by initiating a cascade of reactions upon binding to the cell surface, or have separate external and internal sites of action. The discovery of cyclic AMP by Sutherland and his colleagues⁸⁰, and the finding that peptide hormones regulate cyclic AMP levels, gave impetus to the notion that peptide hormones act at the cell surface and do not need to enter cells. Instead, hormones were postulated to act on the surface of cells to raise or lower the levels of small intracellular messenger molecules such as cyclic AMP or cyclic GMP. However, recently a variety of studies have indicated that insulin enters fibroblastic cells in endocytic vesicles⁸¹ and that there might be specific nuclear receptors for insulin⁸² and other peptide hormones. In considering the mechanism of action of the *src* gene product, one is struck by the variety of effects that develop upon expression of a single gene and this has led us to wonder how a single gene product can have its diverse effects on cell growth, cell shape and the synthesis of specific proteins. Has *src* a single site of action or has it multiple sites of action? Perhaps the convergence of endocrinology and tumour virology will enable us to answer this question and lead to some new insights on the biochemical mechanisms by which growth factors act to control cell growth.

Fig. 5 Scheme of changes in RSV transformation that contribute to the 'morphologic phenotype'. Possible 'other changes' in membrane function could be alterations in calcium permeability or localised increases in protease activity.



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articles

Average properties of X-ray burst sources

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Observations of X-ray bursts from 10 sources reveal a remarkable uniformity in the properties of X-ray burst sources: if the peak luminosity in a burst is a standard candle the effective black body radius observed in bursts, is the same for all burst sources.

THE observed properties of X-ray burst sources have been reviewed by Lewin^{1,2} and by Lewin and Joss³. Most theoretical models of these objects can be put into one of the following two categories: (1) models in which the accretion flow on to a compact object occurs in spasmodic bursts which produce corresponding bursts of X-ray emission. The compact object may be a white dwarf, neutron star or black hole³. (2) Models in which the X-ray burst is caused by a thermonuclear flash in the freshly accreted material on the surface of a neutron star^{4–7}. The flash occurs when critical values of the temperature and pressure at the base of the envelope are exceeded. Hoffman, Marshall and Lewin¹⁰ distinguished two types of X-ray bursts. The type II bursts, repeating on time scales of seconds to minutes, are emitted by MXB1730–335 (rapid burster) only. Type I bursts are emitted by all burst sources, they repeat on time scales of hours and longer, and show a spectral softening as they decay. It is these type I bursts which are studied here.

The average properties of X-ray bursts, specifically their total integrated fluxes, maximum fluxes and spectra, are analysed and discussed here. It is shown that the X-ray bursts sources display a striking uniformity in their observed properties. With the assumption that the peak luminosity of X-ray bursts is a standard candle, this observed uniformity implies that the tails of X-ray bursts are emitted by cooling surfaces

whose size is the same for all burst sources (to within $\pm 20\%$). Also the total energy emitted from X-ray bursts is the same for all burst sources (to within $\pm 30\%$).

Observations

Extensive data on the X-ray bursts from 10 sources have been obtained using the SAS 3 X-ray observatory. Part of the data has been described elsewhere^{8–16}. In the present study the only bursts considered were those observed with the satellite's Y-axis horizontal tube detectors and for which an accurate determination of the position of the X-ray burst source relative to the field of view was available. Background-corrected count rates were obtained in five energy channels between ~ 1.3 and ~ 30 keV. The conversion from count rates to energy fluxes was made with a calibration of the detector sensitivities derived from observations of the Crab nebula.

The following properties of each X-ray burst were evaluated: maximum flux, $F_{\max}$; integrated energy flux, E_b ; time variation of the flux F and the spectral shape of the X-ray emission during the slowly decreasing later parts of the burst, that is, the burst 'tail'. Such tails were first observed by Lewin *et al.*¹⁴ in the bursts of MXB1743–29.

To improve the statistical accuracies I determined the average flux variation of several bursts from each of the sources. From most sources the X-ray bursts are sufficiently similar to make this acceptable. The bursts were aligned at the moment of their initial rise. In the case of MXB1837+05 the variability of the initial peak of the burst is appreciable. From this source the bursts were, therefore, aligned at a point where the initial peak has terminated and the decay of the burst in a 'tail' could be first discerned.

Table 1 gives the average values of $F_{\max}$ and E_b , and Table 2 contains the observed average flux in several consecutive segments of the tails of the burst for each source. Depending on

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Table 1 Average properties of X-ray burst sources

Source (MXB)	$F_{\max}$ (10^{-8} erg cm $^{-2}$ s $^{-1}$)	E_b (10^{-7} erg cm $^{-2}$)	$E_b/F_{\max}$ (s)	No. of bursts
1636-53	7.3 ± 1.3	5.0 ± 0.7	6.9 ± 2.2	4
1659-29	1.2 ± 0.4	1.5 ± 0.2	11.9 ± 3.5	5
1728-34	8.1 ± 0.6	6.7 ± 0.7	8.3 ± 1.5	9
1735-44	2.9 ± 0.2	1.3 ± 0.1	4.3 ± 0.6	19 (May 1977)
	2.8 ± 0.3	1.3 ± 0.1	4.7 ± 1.0	9 (June 1977)
1742-29	2.7 ± 0.4	1.8 ± 0.3	6.8 ± 1.9	4
1743-28	2.3 ± 0.6	2.1 ± 0.7	9.3 ± 5.1	3
1742-29	4.0 ± 0.4	4.9 ± 0.6	12.2 ± 2.7	3
1837+05	3.3 ± 0.2	1.6 ± 0.1	5.0 ± 0.6	15 (July 1976)
	2.7 ± 0.3	1.6 ± 0.2	5.9 ± 1.3	8 (June 1977)
1906+00	2.5 ± 0.5	1.6 ± 0.1	6.2 ± 1.6	7
1916-05	2.1 ± 0.2	1.3 ± 0.1	7.1 ± 0.8	6

the available number of counts the time intervals in these segments vary from 4.2 to 16.6 s. Mean errors are given, as determined from the scatter of the values for individual bursts of one source around the average for that source. This scatter of the total burst energy and the maximum flux is less than 40% for all sources, and typically amounts to between 20 and 30%.

The variability of these burst properties seems small enough to make a worthwhile analysis of X-ray burst sources feasible on the basis of average burst properties.

Burst tails and maximum flux of X-ray bursts

Swank *et al.*¹⁹ showed that the shape of the X-ray spectrum in the tail of one burst observed from a particular source near $\alpha = 261^\circ$ and $\delta = -31^\circ$ is best described by a Planck function, with a decreasing temperature T . Similar results have been obtained for the source MXB1728-34 and MXB1636-53 (refs 11, 12). For the latter sources it was found that the flux in the tail of bursts is $\sim$ proportional to T^4 . These results strongly suggest that the radiation in the tail of a burst is emitted by a cooling surface of constant size.

To pursue this idea further we fit black-body spectra with absorption cutoffs to the X-ray data in sequential segments of

the composite bursts of each of the 10 sources. The best-fit values of the temperature T and the interstellar hydrogen column density N_H are given in Table 2.

The observed temperature T and flux F of a black body determine the solid angle which the radiating surface subtends. To find the size of this surface we must know its distance, and we also have to make an assumption about its geometry. If we assume that the surface is spherical, the relationship between its radius R and distance d is given by $R^2 = d^2 F / \sigma T^4$ (σ is the Stefan-Boltzmann constant). (The spectral data do not exclude the possibility that the spectrum is actually that of a grey body: $F_\nu = c B_\nu$, with $c < 1$, independent of frequency. This possibility has been suggested in the context of accretion instability models of X-ray burst sources³⁷. In this case the derived solid angle subtended by the radiating surface is a lower limit to its actual value.)

Guided by the notion that the peak luminosity of X-ray bursts might provide a standard candle (that is, $d \propto F_{\max}^{-1/2}$) I have determined for each source the observed compound quantity $T^{-2}(F/F_{\max})^{1/2}$ for different parts of the burst tails; these are also given in Table 2.

Two results clearly emerge: (1) for each source the value of $T^{-2}(F/F_{\max})^{1/2}$ does not change significantly throughout the

Table 2 Observed flux, temperature and interstellar hydrogen column density in tails of X-ray bursts

Source (MXB)	F (10^{-8} erg cm $^{-2}$ s $^{-1}$)	kT (keV)	$(kT)^{-2}(F/F_{\max})^{1/2}$	N_H (10^{22} cm $^{-2}$)
1653-36	2.13 ± 0.28	1.85 ± 0.05	0.158 ± 0.033	0.1 ± 0.3
	0.54 ± 0.08	1.08 ± 0.09	0.233 ± 0.076	1.6 ± 0.9
	0.42 ± 0.07	1.08 ± 0.10	0.207 ± 0.074	2.2 ± 1.1
1659-29	0.74 ± 0.19	2.00 ± 0.16	0.150 ± 0.066	1.4 ± 1.2
	0.44 ± 0.09	1.72 ± 0.13	0.120 ± 0.038	0.6 ± 0.8
	0.28 ± 0.06	1.53 ± 0.11	0.095 ± 0.029	0.6 ± 0.7
1728-34	5.20 ± 0.66	2.33 ± 0.04	0.147 ± 0.020	4.9 ± 0.5
	1.48 ± 0.17	1.54 ± 0.05	0.180 ± 0.028	6.0 ± 0.7
	0.42 ± 0.04	1.09 ± 0.08	0.191 ± 0.047	6.8 ± 1.6
1735-44	0.39 ± 0.03	1.51 ± 0.12	0.161 ± 0.038	0.9 ± 0.9
	0.14 ± 0.02	1.01 ± 0.29	0.217 ± 0.148	1.2 ± 2.4
	0.48 ± 0.07	1.76 ± 0.12	0.134 ± 0.035	-0.1 ± 0.8
1735-44	0.18 ± 0.02	0.99 ± 0.28	0.261 ± 0.177	1.3 ± 2.5
	1.10 ± 0.14	2.01 ± 0.25	0.158 ± 0.060	5.0 ± 3.6
	0.35 ± 0.03	1.62 ± 0.58	0.137 ± 0.113	9.0 ± 13.4
1742-29	1.08 ± 0.12	1.59 ± 0.30	0.273 ± 0.151	10.7 ± 8.2
	0.41 ± 0.16	0.95 ± 0.48	0.771 ± 0.625	19.7 ± 28.4
	3.45 ± 0.71	2.35 ± 0.17	0.167 ± 0.050	14.6 ± 4.9
1743-29	0.91 ± 0.16	1.78 ± 0.25	0.150 ± 0.063	12.0 ± 6.8
	0.69 ± 0.43	1.65 ± 0.24	0.152 ± 0.100	11.0 ± 6.4
	0.52 ± 0.06	1.60 ± 0.12	0.157 ± 0.037	-0.2 ± 0.7
1837+05	0.17 ± 0.04	1.04 ± 0.26	0.209 ± 0.133	2.1 ± 2.8
	0.55 ± 0.08	1.90 ± 0.15	0.124 ± 0.036	-0.5 ± 1.0
	0.20 ± 0.05	1.30 ± 0.25	0.162 ± 0.093	1.1 ± 2.2
1906+00	0.57 ± 0.05	1.42 ± 0.10	0.236 ± 0.069	0.9 ± 0.7
	0.21 ± 0.02	1.09 ± 0.20	0.242 ± 0.126	1.0 ± 1.7
	0.51 ± 0.04	1.57 ± 0.13	0.201 ± 0.048	2.1 ± 1.2
1916-05	0.11 ± 0.02	1.22 ± 0.33	0.153 ± 0.102	-0.7 ± 1.6

burst. This supports an earlier conclusion¹¹ that we observe a cooling surface of constant size in the tail of an X-ray burst. (2) The average of $T^{-2}(F/F_{\max})^{1/2}$ has about the same value for all burst sources in the present sample. The distribution of their average values has a standard deviation of only 20%.

I suggest that this apparent uniformity in the observational data is not a fortuitous coincidence, but that it reflects the underlying unity of X-ray burst sources: if the peak luminosity of X-ray bursts is, indeed, a standard candle, this observation implies that all compact objects giving rise to X-ray bursts have approximately the same size (and possibly mass).

The existence of a fixed upper limit to the X-ray burst luminosity is suggested by the fact that the peak luminosity of bursts from a given source does not vary by more than a few tens of per cents, indicating that there is a limit imposed on the maximum attainable luminosity in X-ray bursts. Furthermore, in spite of their strongly different properties and presumably different origin there is no systematic difference in the maximum luminosity of type I and type II bursts¹⁰ emitted by the rapid burster (MXB1730–335). This limit is reached by the type I bursts, and by all large type II bursts. This result indicates that the upper limit is independent of the mechanism causing the X-ray bursts, but is determined by global properties of the system. In this respect it should be noted that the numerical calculations by Joss^{5,20} show that if the X-ray bursts are caused by thermonuclear flashes on the surface of a neutron star, then their peak luminosity is, to within a factor of order unity, given by the Eddington limit.

Discussion

I will explore the consequences of assuming that the peak luminosity in X-ray bursts is determined by the Eddington limit $L_E = 1.25 \times 10^{38} (M/M_\odot) \text{ erg s}^{-1}$. First, for the globular cluster burst sources MXB1820–30 (refs 21, 22, 24), 0512–40 (ref. 23) and 1703–335 (refs 18, 25, 27) (rapid burster) this assumption allows a rough estimate of the mass of the X-ray star. The maximum fluxes in the bursts from these sources (ref. 22 and F. Li and H. Marshall, personal communications) and the optically determined distances to the globular clusters^{24–27} in which they are located then give masses of 2.4, 1.4 and 2.6 $M_\odot$, respectively. In view of the uncertainty of the distances (especially that of MXB1730–335) these numbers cannot be taken at face value; however, they clearly indicate compact objects of roughly solar mass.

Second, from the assumption that the peak luminosity of an X-ray burst is determined by the Eddington limit L_E it follows that the total energy in a burst equals $E_b L_E / F_{\max}$. The ratio $E_b / F_{\max}$ (the 'effective duration' of the burst) is known to be approximately the same for all burst sources². Table 1 gives the average values of $E_b / F_{\max}$ for the sources studied here. The distribution of these average values has a standard deviation of only 35%. This implies that the average total burst energy is approximately the same for all burst sources (see Table 3). Unless bursts with a significantly shorter or longer duration are systematically much weaker than those from any of the burst sources studied here, this result does not seem to be the consequence of a selection effect.

If X-ray bursts originate from black holes, the apparent uniformity in the observed properties does not have an easy interpretation. There are no obvious evolutionary reasons for a particular value of the black-hole mass (and therefore the Eddington limit). In fact, black-hole theories of X-ray bursters² have used masses ranging from ~ 10 to a few thousand solar masses. For this reason, but without implying that the present observations conflict with black-hole models, I will concentrate on the consequences of the assumption that the compact objects in X-ray burst sources are neutron stars.

Available mass determinations of neutron stars in young massive X-ray binaries cluster around 1.4 $M_\odot$ (ref. 28). Because their galactic distribution is characteristic of a population II type of object it is very unlikely that X-ray burst sources are members of such young systems². Indeed, in those

Table 3 Distance, radius and total burst energy of X-ray burst sources

Source (MXB)	Distance (kpc)	Radius (km)	Total burst energy (10^{39} erg)
1636–53	4.5 ± 0.4	7.4 ± 1.2	1.20 ± 0.38
1659–29	10.9 ± 1.6	4.5 ± 1.0	2.09 ± 0.73
1728–34	4.2 ± 0.2	6.5 ± 0.4	1.45 ± 0.26
1735–44	7.2 ± 0.2	7.2 ± 1.6	0.80 ± 0.10
1742–29	7.4 ± 0.5	5.5 ± 1.8	1.19 ± 0.33
1743–28	8.0 ± 1.0	13.9 ± 9.5	1.62 ± 0.89
1743–29	6.0 ± 0.3	5.8 ± 1.0	2.12 ± 0.47
1837+05	7.0 ± 0.4	6.1 ± 1.1	0.96 ± 0.13
1906+00	7.6 ± 0.8	8.9 ± 1.9	1.09 ± 0.28
1916–05	8.4 ± 0.3	6.6 ± 1.8	1.07 ± 0.14

cases where their optical counterparts have been identified plausibly, the optical objects are stars of low mass. Nevertheless, the masses of the neutron stars are probably not much different from 1.4 $M_\odot$. Many X-ray burst sources are associated with a source of steady X-ray emission², and clearly belong to the galactic bulge population. Based on their spatial distribution, the absence of observed X-ray eclipses, and the lack of bright optical counterparts, Joss and Rappaport²⁹ have proposed that bulge sources are low-mass binary systems consisting of a neutron star ($\sim 1.4 M_\odot$) and a low-mass companion ($\leq 0.3 M_\odot$). Such a system could evolve³⁰ from a cataclysmic variable in which the white dwarf component becomes more massive than the Chandrasekhar limit due to mass accretion. It then collapses into a neutron star, with mass $\sim 1.2 M_\odot$. Allowing the newly formed neutron star to accrete, at most, the remaining mass of its companion, its mass will not differ from 1.4 $M_\odot$ by more than 0.5 $M_\odot$ (ref. 32).

Values of the distance and radius of the X-ray burst sources derived from equating their maximum burst luminosity to the Eddington limit of a 1.4 $M_\odot$ object, are given in Table 3. For a mass M , the results are proportional to $M^{1/2}$. The error estimate includes both the uncertainty of the individual values of $T^{-2}(F/F_{\max})^{1/2}$ and the standard deviation of their distribution.

Most of the X-ray burst sources are located in the galactic bulge, consistent with their galactic longitude distribution². In particular, two of the three galactic centre (GC) sources¹⁴ are at distances compatible with their position at the centre of the Galaxy. Their values of N_H are consistent with the low-energy cut off in the spectrum of the extended steady source GCX (refs 14, 31). Their interstellar extinction A_V , as obtained from N_H (refs 33, 34), is between 30 and 50 mag. These results support the conclusion that the three GC burst sources and the steady source GCX are actually near the centre of the Galaxy, for which A_V is at least 28 mag (ref. 36).

Only one of the sources in the present sample is at the far side of the Galaxy. Bursts like those of MXB1636–53 and MXB1728–34 would still be detectable from a source at a distance of ~ 15 kpc. This indicates that the peak luminosity of X-ray bursts may be somewhat higher than the value taken here. Furthermore, if there were an observational bias it is expected to favour the closer sources (easier discovery, and possible influence on the choice of observing programs).

Except for the large, but very uncertain, result on MXB1743–28, the average value for the radius of the radiating surface derived here, equals 6.5 km, with a standard deviation of 1.3 km. In view of the uncertainty of the individual radius determinations this result is consistent with an identical value of ~ 7 km for all burst sources in the present sample.

If X-ray bursts originate from the surface of neutron stars, the following arguments (admittedly model dependent) suggest that the radius of the radiating surface may be possibly identified with the radius of the neutron star.

First, in spite of much observational effort no periodic variability, reminiscent of X-ray pulsars, has been found in any of the steady bulge sources. This may indicate that magnetic fields

are not present in these systems. Thus the accreted matter probably spreads uniformly over the surface of the neutron star. Second, if the X-ray burst is caused by a thermonuclear flash, the amount of energy available per particle is insufficient to lift the envelope by more than a few per cent of the neutron star's radius.

The possibility of deriving the radius of a neutron star, along the lines described here, offers in principle an opportunity to put conditions on the theories of matter at densities above $3 \times 10^{14} \text{ g cm}^{-3}$. The numerical value for the radius of a neutron star, derived here, may be wrong by a systematic factor, due mainly to the assumption that the maximum luminosity in an X-ray burst is exactly equal to the Eddington limit, the assumed value for the mass of the neutron star and the neglect of the influence of a strong magnetic field on the distribution of the radiating material on its surface.

Until more theoretical and observational information on these aspects is available and our uncertainty on the origin of X-ray bursts has disappeared it seems premature to confront the observed radius of $\sim 7 \text{ km}$ with predictions from theoretical models of neutron stars. However, if for the time being we take the numbers derived here, at face value, there seems to be no conflict with the theoretical radii, for which (depending on the equation of state of the high-density matter) values ranging from ~ 5 to $\sim 15 \text{ km}$ have been given³⁵.

Many of the observed properties of X-ray burst sources (peak luminosity, total integrated flux, burst intervals, black-body temperatures) can be explained by the thermonuclear flash model²⁰. The predicted ratio of the steady X-ray luminosity to the time-averaged burst luminosity (a few hundreds) is in reasonable agreement with the observed values for most of the burst sources, except MXB1730–335 (rapid burster) and MXB1659–29 (ref. 2).

For the rapid burster, a solution to this problem has been proposed by Hoffman, Marshall and Lewin¹⁰; in this source the steady emission occurs in the form of rapid type II bursts. The ratio of the time-averaged luminosity in these type II bursts to that in type I bursts (~ 130) (ref. 10) is consistent with the thermonuclear flash model²⁰.

For MXB1659–29 a solution to the problem might be that in this particular case the neutron star has a small mass and—

according to almost all neutron star models³⁵—a large radius. The present results on MXB1659–29 do not support this interpretation: if there is any difference in the radius for this source at all, it is smaller than for the other burst sources. (The particular choice of $1.4 M_{\odot}$ for the neutron star mass does not cause this result: with a lower mass the distance, and therefore the radius, would become even smaller.)

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Tectonic controls of late Cretaceous sedimentation, western interior, USA

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Anomalous subsidence of continental crust occurred during the Late Cretaceous in a region centred about Colorado, southern Wyoming and eastern Utah. The amount of subsidence is greater than can be explained by eustatic sea level rise and supracrustal loading by water and sediment. Analysis of thickness and distribution of Upper Cretaceous strata of the Western Interior Seaway, USA, shows two distinct basinal trends. The subsidence of the earliest (100–80 Myr BP) is attributed partly to isostatic flexure in front of the rising orogenic belt. The subsidence of the later (80–70 Myr BP) is attributed to subcrustal loading induced by a shallowly subducted plate.

TECTONIC controls of subsidence, patterns of sedimentation and subsequent evolution of major basins associated with active plate boundaries have received much attention in recent years. In particular, application of plate tectonic concepts and studies of sediment distribution in major basins along contemporary active plate margins have provided analogues for interpreting formation of ancient basins which occupied sites along or near ancient active plate boundaries. In contrast, the controls of subsidence of regions within cratonic areas which are surrounded by largely anorogenic terranes and located at great distances from known coeval active plate boundaries, are poorly understood.

Here we review the evidence which demonstrates that a broad region of the late Cretaceous seaway of North America, centred about Colorado, southern Wyoming and eastern Utah, was the site of subsidence and sediment accumulation of

greater magnitude than can be explained by isostatic response to supracrustal loading. Analysis of sediment thickness and distribution of Upper Cretaceous strata deposited during short time intervals further shows that subsidence within this region occurred in two distinct phases with corresponding disparity in amount, position and geometry of subsidence. From 100 to 80 Myr BP, anomalous subsidence was restricted to a narrow trough elongate in a north-south direction and colinear with the axis of the Sevier orogenic belt. From 80 to 70 Myr BP, the locus of maximum subsidence shifted to a broader region, subcircular in outline, where about 3 km of sediment accumulated. This observed shift in position and geometry of sedimentary basins and, particularly, the development of the latter phase of regional subsidence are not explained satisfactorily by commonly proposed mechanisms of subsidence within dominantly anorogenic continental regions underlain by continental crust of normal thickness.

Two mechanisms, subcrustal cooling and displacement of asthenosphere by denser lithosphere, are proposed to explain the development of the broad depression and thick sedimentary prism during the late Cretaceous. The mechanisms are derived by geophysical modelling of the amount and nature of subsidence of continental lithosphere immediately underlain by oceanic lithosphere which is colder and slightly denser than the asthenosphere it displaces. We recognise a temporal and spatial correspondence in the development of the regional depression and an inferred shallowly subducted oceanic plate. Empirical support for this interpretation is presented in a brief analysis of tectonic and topographic characteristics of Peru which provides a contemporary analogue of the late Cretaceous events in the Western Interior of the USA. Subsequent uplift of the Rocky Mountains during Laramide tectonism is related, in part, to isostatic rebound which occurred as the subducted plate detached from the base of the overlying continental lithosphere.

Sea-level rise and continental submergence

One of the most extensive and best documented periods of sea-level rise and continental submergence on a global scale began during the mid-Albian (~100 Myr BP) and ended by the late Maestrichtian (65 Myr BP) (ref. 1). Estimates of the actual amount of sea-level rise range from 200 m to more than 500 m and the transgression has been attributed to simultaneous epeirogenic subsidence of several continents, to eustatic sea-level rise, or to both. The history of transgression is well documented in the Western Interior Seaway of the USA and Kauffman² has summarised the history of minor transgressive and regressive fluctuations in the dominant pattern of late Cretaceous flooding of that area. Bond³ estimated that a relative rise in sea level of 310 m is required to flood the area of North America submerged during the late Cretaceous transgression. Bond determined that crustal subsidence and, thus, maximum sediment thickness of ~700 m would result from supracrustal loading induced by a 310-m rise of sea level. Bond observed that Upper Cretaceous strata in as much as half of the North American continent are more than 700 m thick and concluded that some tectonic process, other than supracrustal loading, was responsible for the observed subsidence.

It may be significant that Bond's estimate of crustal subsidence resulting from a eustatic sea-level rise of 310 m is conservative as is his estimate of total Upper Cretaceous stratigraphic thickness. Bond relied principally on Cretaceous isopach maps of the Shell Oil Company atlas⁴ which depict existing stratigraphic thickness and, thus, underestimate depositional thickness. In addition, if actual eustatic sea-level rise relative to the North American continent was >310 m, say 400 m, the maximum subsidence due to supracrustal loading would approach 1 km. The important point is that although we agree with Bond's conclusion that the amount of subsidence and thickness of accumulated sediment in a portion of the Western Interior Seaway cannot be explained solely by

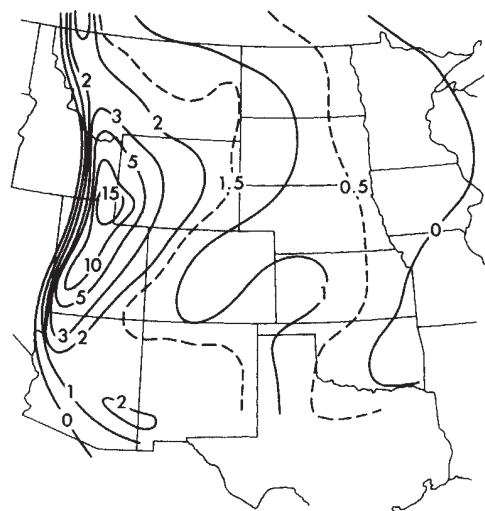


Fig. 1 Restored thickness of Upper Albian through Santonian strata. Isopach contours in 10^3 ft.

supracrustal loading, we consider that portion to be limited to the Colorado-Wyoming-eastern Utah (CWU) locus of maximum subsidence. This conclusion is supported by the observation that Upper Cretaceous strata thicken gradually away from the zero isopach and, with the exception of anomalously thick strata in the CWU locus, have a maximum thickness of about 1 km throughout the Western Interior Seaway.

History of subsidence

To understand the spatial and temporal history of subsidence and to interpret the cause or causes of subsidence, apart from supracrustal loading, we relied principally on published isopach maps of strata representing each Upper Cretaceous stage⁵. These maps, which are compatible with more recent regional syntheses of Cretaceous stratigraphy^{4,6-8}, represent restored depositional thicknesses (exclusive of compaction) across areas where Upper Cretaceous strata have been removed by post-Cretaceous uplift and erosion. Stratigraphic thicknesses portrayed on each map were verified or modified by consulting more than 150 other reports on Upper Cretaceous stratigraphy dealing with more local scales of study. When available, biostratigraphic ages of marine strata within each stage were related to the Cretaceous radiometric time scale for the Western Interior². Otherwise, stratigraphic thicknesses were assigned to a particular stage as published. Although such reconstructions are ambiguous and generalised, sufficient control exists to document the first hypothesis of this report; that maximum subsidence greater than can be explained by isostatic response to supracrustal loading occurred in two distinct areas during two intervals of time.

On the maps prepared for each stage, isopach patterns, thickness maxima and inferred loci of maximum subsidence are remarkably similar for Upper Albian (~98 Myr BP) to Santonian (82 Myr BP) strata. By contrast, Campanian and Maestrichtian (82–65 Myr BP) strata display a distinctly different isopach pattern and a shift in position of maximum thickness and, presumably, greatest subsidence. As a means of summarising the change in position and pattern of subsidence which occurred at ~80 Myr BP in the Western Interior Seaway, isopach maps of Upper Albian through Santonian and Campanian–Maestrichtian strata are presented in Figs 1 and 2, respectively. Each map was constructed by placing a grid (200 points) over the isopach maps of each stage, obtaining the thickness for each point by interpolation, and contouring the cumulative thickness on the resultant grid network. This method results in considerable loss of detail of history of sediment accumulation and evolution of individual basins through

time. However, it has the advantage of using all data from incomplete or incompletely measured stratigraphic sections and of averaging total thicknesses over a broad region to obtain the gross character of isopach trends and positions of maximum thickness.

Strata of Upper Albian to the Santonian age are thickest along a north-south trending depositional trough aligned parallel to the Sevier orogenic belt. To the east, stratigraphic thickness diminishes quickly and the remainder of the Western Interior Seaway appears as a broad and elongate region of subsidence and sedimentation (Fig. 1). Thick wedges of conglomerates, sandstones and minor siltstones derived from the Sevier highlands were deposited in the rapidly subsiding trough, whereas marine shale and minor intercalated siltstone and limestone dominate the rest of the Interior Seaway. Paralic marine and fluvial deposits are restricted to a narrow band coincident with the eastern flank of the Sevier orogenic belt.

Deposition of Campanian and Maestrichtian sediment occurred throughout most of the Western Interior Seaway⁶ but greatest accumulation was centred about a broad region in southern Wyoming, central and northern Colorado, and eastern Utah. The isopach map of these strata (Fig. 2) does not show the development of a linear depositional trough as occurred during the earlier part of the late Cretaceous. Rather, subsidence seems to have been a regional, subcircular downwarp with isolated basins in southern Wyoming, northwestern and northcentral Colorado and eastern Utah. Palaeo-environmental interpretations indicate that a broad fluvial-deltaic and paralic sediment plain developed along the western margin of the Western Interior Seaway after the beginning of the Campanian. These strata interface with marine shale and siltstone and minor intercalated limestone and calcareous mudstone of the broad epicontinental sea to the east. During the Campanian, fluvial, deltaic and coastal plain systems prograded eastwards across marine strata such that by the end of the Campanian (~69 Myr BP) the area of marine deposition was greatly restricted. Deposition of predominantly fluvial sediment during the Maestrichtian completed the regression of the Cretaceous sea. The change in character and position of depositional facies at the Campanian-Maestrichtian boundary is significant in that, in all probability, they reflect a change in tectonic controls of subsidence. Campanian strata in the CWU locus are predominantly marine and the thickness accounts for about two-thirds of the total thickness shown on Fig. 2. Maestrichtian strata are comparatively thin and primarily non-marine or paralic. Thus, we infer that the depositional pattern and the regional subsidence of the CWU locus shown in Fig. 2 were created almost entirely during the Campanian, that is, from ~80 to ~70 Myr BP.

Subsidence of intra- or pericratonal basins

Three categories of mechanisms are commonly proposed to explain subsidence of intra- or pericratonal basins. These are: (1) gravitational loading; (2) thermal decay and crustal contraction; and (3) stress-induced subsidence. Gravitational mechanisms invoke either global sea-level rise to initiate supracrustal loading and subsidence of broad, topographically low regions of cratons or subsidence along rifted plate margins which have crustal thickness and density that is transitional between normal continental and oceanic crust. In the former instance, subsidence is limited to ~2.4 times the initial water depth and, as discussed previously, this mechanism does not account for the amount of subsidence which occurred during the late Cretaceous in the CWU locus. Thermal mechanisms involve subcrustal loading induced by some thermal event such as magmatism, asthenospheric diapirism and crustal thinning, or intrusion of basic or ultrabasic material into the lower part of the crust. Slow subsidence at an exponentially decreasing rate results from thermal decay and contraction of lithosphere subsequent to an initial thermal event (crustal heating and uplift). The amount of subsidence by such mechanisms is limited to the amount of material removed by surficial or

subcrustal erosion during the initial stage of uplift. No one has suggested and there is no evidence to indicate that late Cretaceous subsidence in the CWU locus is the result of such a mechanism. Subsidence and basin formation through some tectonic or structural deformational event, the third category, are not indicated by the regional nature of subsidence during the Campanian. Subsidence along the north-south trending trough during the Albian until the Santonian, however, is clearly related to deformation along the Sevier orogenic belt.

The inadequacy of these conventional mechanisms to explain the broad, regional subsidence during the Campanian has led us to examine the late Cretaceous tectonic setting of the western USA in an attempt to define other possible origins of subsidence. We have previously summarised⁹ the evidence which suggests that during the Campanian the Farallon plate was subducting at a shallow angle beneath the central part of the western USA. The inferred geometry of this episode of shallow subduction, places the oceanic lithosphere in virtual contact with overlying continental lithosphere. The timing of the inferred period of shallow subduction is constrained by the end of the subduction-related volcanism and plutonism in the Sierra Nevada (~80 Myr BP) and subsequent initiation of magmatism in Colorado at ~70 Myr BP. Similarly, the position of the shallowly subducted lithosphere is constrained by the position of the magmatic gap which is coincident with the limits of regional subsidence in the CWU locus, as inferred from the preserved sedimentary record. This history is shown in Fig. 3.

Mechanisms of regional subsidence

Spatial and temporal correspondence between low angle subduction and regional subsidence in the CWU locus suggests a genetic relationship. We have modelled the effects of low angle subduction on subsidence of overlying lithosphere and find that regional subsidence equal to or greater than that observed is induced by two mechanisms: isostatic subsidence by subcrustal loading and subcrustal cooling.

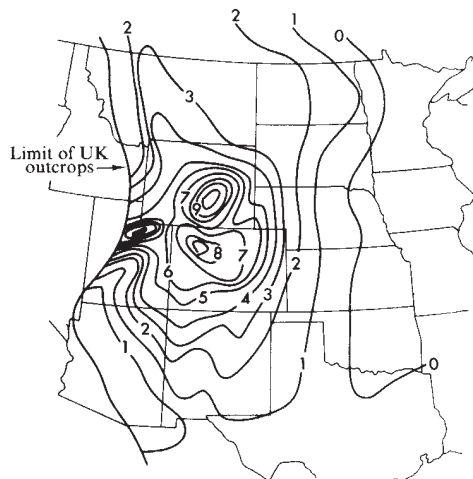


Fig. 2 Restored thickness of Campanian and Maestrichtian strata. Isopach contours in 10^3 ft.

The first of these, isostatic subsidence by subcrustal loading, results from replacement of asthenosphere by slightly denser subducted oceanic lithosphere. Contemporary examples of shallow subduction are associated with underthrusting of aseismic ridges on oceanic lithosphere^{10,12}. The increased crustal thickness of the ridges reduces the average density of the lithosphere by $\sim 0.05 \text{ g cm}^{-3}$ (from 3.24 to 3.19 g cm^{-3}) and, therefore, reduces the density contrast with the asthenosphere^{10,12}. Consequently, gravitational instability of the subducting lithosphere is reduced or eliminated, the subducting plate is more buoyant and descends at a shallow angle, and asthenosphere is displaced by slightly denser lithosphere. In addition, through the derivation of a density model of the

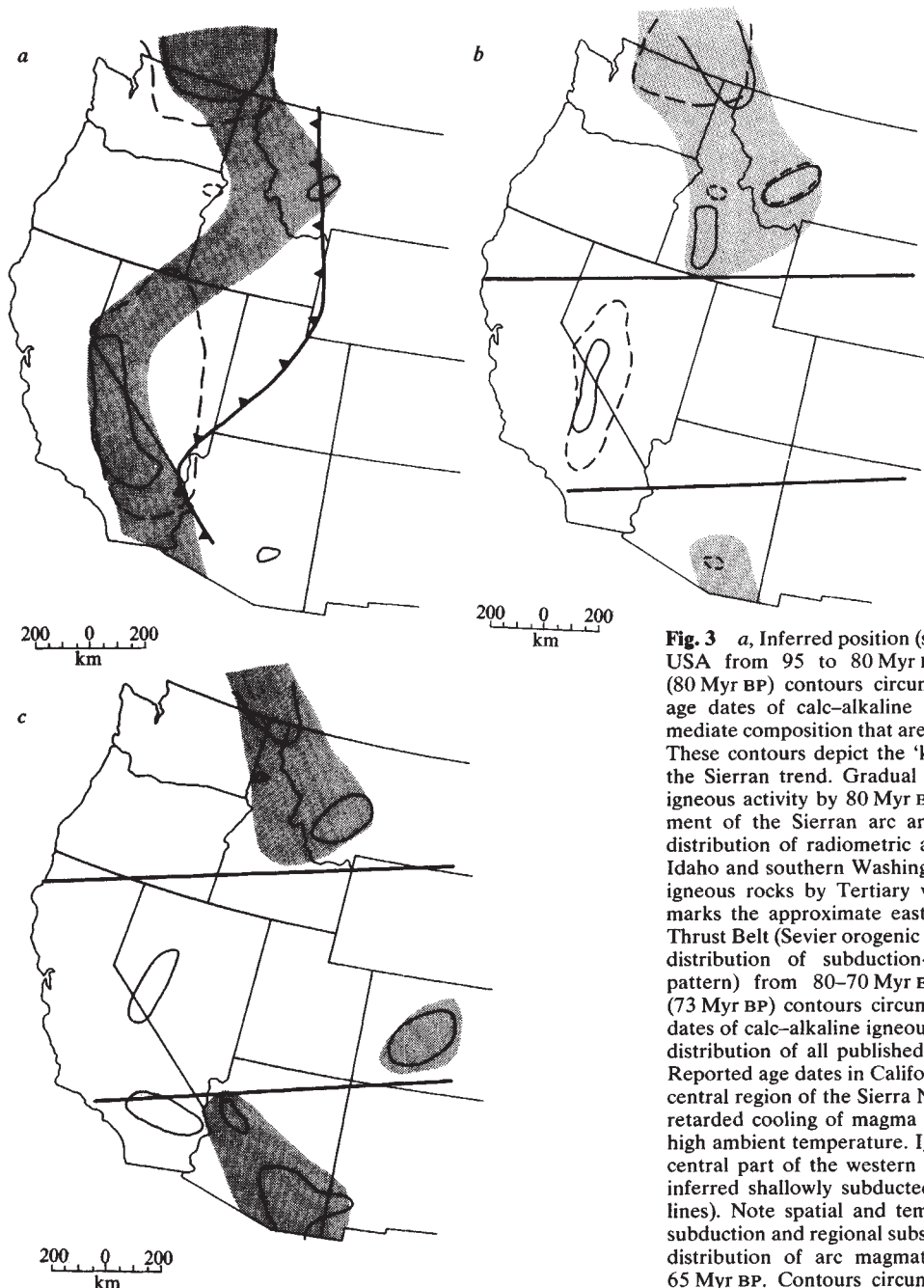


Fig. 3 *a*, Inferred position (stippled) of Sierran arc in the western USA from 95 to 80 Myr BP. Solid (95 Myr BP) and dashed (80 Myr BP) contours circumscribe all isochronous radiometric age dates of calc-alkaline plutonic rocks of silicic and intermediate composition that are grouped within well defined regions. These contours depict the 'known' positions of plutonism along the Sierran trend. Gradual reduction and inferred cessation of igneous activity by 80 Myr BP along the California-Nevada segment of the Sierran arc are indicated by the more restricted distribution of radiometric age dates. Sparse dating in Oregon, Idaho and southern Washington reflects extensive cover of older igneous rocks by Tertiary volcanics. Line with thrust symbols marks the approximate eastern limit of the Foreland Fold and Thrust Belt (Sevier orogenic belt in Utah and Nevada). *b*, Inferred distribution of subduction-related igneous activity (stippled pattern) from 80–70 Myr BP. Solid (76 Myr BP) and dashed (73 Myr BP) contours circumscribe isochronous radiometric age dates of calc-alkaline igneous rocks and are representative of the distribution of all published age dates within this time interval. Reported age dates in California and Nevada are restricted to the central region of the Sierra Nevada batholith complex and reflect retarded cooling of magma emplaced into a region of relatively high ambient temperature. Igneous activity was suspended in the central part of the western USA and this region lies above the inferred shallowly subducted oceanic plate (indicated by heavy lines). Note spatial and temporal correspondence of low angle subduction and regional subsidence in the CWU locus. *c*, Inferred distribution of arc magmatism (stippled pattern) from 70 to 65 Myr BP. Contours circumscribe regions in which 70 Myr BP isotopic age dates of igneous rocks have been reported. A few isolated dates in California and Nevada are interpreted as delayed cooling ages of rocks in the central and hotter portion of the extinct Sierran arc. Laramide deformation and igneous activity began at 70 Myr BP in Colorado; the magmatic null persisted to the west above the region of low angle subduction.

central Andean subduction zone, Jacoby¹³ suggested that in normal subduction a zone of anomalously low density is present between the descending slab and the base of the overlying continental crust, whereas in low angle subduction we suggest that this wedge of low density asthenosphere is displaced by subducted lithosphere.

Assuming isotasy, shallow subduction of oceanic lithosphere of either normal or anomalously low density results in subsidence of overlying continental lithosphere. The amount of subsidence expected at the surface of the continent is ~3 km (a more complete discussion of the assumptions and parameters used in the geophysical calculations will be given elsewhere). As subsidence by this mechanism is the result of a displacement phenomenon, it is likely to occur immediately after establishment of low angle subduction. By analogy with contemporary examples of low angle subduction, we presume that late

Cretaceous low angle subduction beneath the western USA was partially induced by subduction of an aseismic ridge on the Farallon plate. Rapid convergence and active overriding of the Farallon plate by North America augmented the shallow angle of subduction^{9,14}.

The second mechanism which would contribute to subsidence of continental crust above a shallowly subducted oceanic plate is subcrustal cooling. Continued low angle subduction of relatively cold oceanic lithosphere will cool the base of the overlying continental crust by conduction^{15,16} and by dehydration of the sinking slab²³. Thus, in addition to displacing anomalously low density asthenosphere following a change from normal to shallow subduction, low angle subduction will also cool any remaining low density, partially molten mantle material. Subcrustal cooling causes increased density of continental crust and hence regional isostatic subsidence in the

region of low angle subduction. Subsidence caused by this mechanism would be slow and would follow the rapid subsidence produced by the first mechanism. Our calculations indicate that ~1–2 km of additional subsidence would occur from subcrustal cooling.

A contemporary analogue for our interpretation of the history and causes of late Cretaceous regional subsidence in the CWU locus may exist in Peru. The Peruvian Andes stand above part of the Nazca plate which is subducting at a shallow angle, in contrast to steeper subduction along most of the remainder of the Peru–Chile trench¹⁷. Furthermore, as Barazangi and Isacks¹⁷ noted, the zone of low angle subduction is, in part, coincident with subduction of the aseismic Nazca ridge. There are no Quaternary volcanoes within this segment of the Andes¹⁸, although radiometric ages of extrusives as young as 5 Myr BP have been reported¹⁹. Although the elevation of the Andean crest in Peru is comparable with the Altiplano region to the south, the Peruvian Andes are remarkably narrower. Coincident with the position of low angle subduction, the headwater drainage system of the broad, low-lying Amazon basin replaces the high, broad uplifted region of the Altiplano. This major topographic depression is predicted by our calculations based on the low angle subduction model.

Conclusions

Isopach maps of late Cretaceous strata display two geometries and positions of maximum thickness and, by inference, maxima of crustal subsidence. The earliest is a north–south trending trough depicted by the isopach of Upper Albian through Santonian strata. Subsidence along this narrow belt is attributed, in part, to lithospheric flexure and isostatic depression induced by the tectonic load of thrust sheets along the front of the rising Sevier orogenic belt. This phase of subsidence will be discussed elsewhere. During the Campanian (from ~80–70 Myr BP), the locus of maximum subsidence shifted to a broad, sub-circular region centred in the CWU locus. Although eustatic sea-level rise, which began in the late Albian, accounts for subsidence in much of the Western Interior Seaway, it cannot explain the anomalous amount of subsidence in the

CWU locus. Through geophysical modelling, comparison with a contemporary analogue, and inferred history of late Cretaceous plate interaction, we interpret the subsidence during the Campanian as a result of low angle subduction. As much as 5 km regional subsidence of crust above a shallowly subducted oceanic plate of anomalously low density is predicted by our calculations. Isostatic subsidence results from subcrustal cooling and displacement of asthenosphere by slightly denser lithosphere. Regional uplift following detachment of the subducted plate from the overlying continental lithosphere is also predicted by our interpretation. Initiation of volcanism, uplift and structural deformation in Colorado at 70 Myr BP, the Laramide orogeny, is partially related to isostatic rebound as a wedge of asthenosphere seeped between the base of the continental lithosphere and the leading edge of the sinking plate. In all probability, isostatic rebound occurred at the same time as other mechanisms proposed for Laramide tectonism^{20–22}.

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Early Precambrian oxygen: a case against photosynthesis

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Recent biological–biochemical and geological–geochemical data are inconsistent with the currently accepted view that early Precambrian fossil microbiotas are evidence of oxygen-generating photosynthesis contributing free oxygen to early Precambrian environments. The alternative is atmospheric photodissociation, perhaps with cyclic or periodic instability.

THE study of Precambrian Earth history presents an enigma. On one hand, it is widely conceded that the primitive Earth was initially devoid of molecular oxygen and that life originated in such an environment. On the other hand, many Precambrian rocks, including the oldest known sediments, contain primary oxidised iron minerals¹ which indicate some source of free oxygen at the time of their precipitation and deposition. Where did this free oxygen come from? The answer to this question

has far-reaching consequences for geology, biology and atmospheric science.

Because volcanic exhalations at present lack free oxygen only two principal sources have been considered plausible for the oxidation of early Precambrian reduced substances. One of these is a biotic source localised in the hydrosphere: oxygen-generating photosynthesis. The other is an abiotic source localised in the atmosphere: photodissociation. Recognising that both processes may have made contributions, most workers have emphasised either the biotic source or the abiotic source as the dominant factor. Macgregor² was among the first to relate photosynthesis to the Precambrian banded iron formations. Cloud^{3–10} has championed the biotic model with support for Margulis *et al.*¹², Schidlowski¹³, and others. Berkner and Marshall¹⁴, Brinkmann¹⁵, and Schopf¹⁶ have favoured photodissociation as the likely source of the early oxygen. At present, the majority opinion seems to favour the biotic explanation. This paper assembles arguments against the photosynthetic

model and in support of the photodissociation model. It is important to emphasise here that this discussion does not concern the origin of the modern oxygenic atmosphere, or even the atmosphere of most of Phanerozoic time, both of which are undeniably the products of green plant photosynthesis.

Early Precambrian oxygen

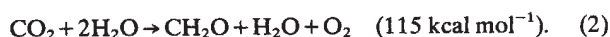
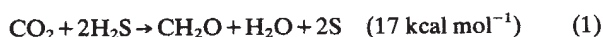
The photosynthetic origin of early Precambrian oxygen carries with it several assumptions, corollaries, and consequences. Among the most important is the implicit assumption that, somehow, oxygen-generating photosynthesis first evolved in an anaerobic organism adapted to an anaerobic hydrosphere. Second, if the oxygen released by this early photosynthesis was to be immediately used in the hydrosphere for the oxidation of ferrous iron (the banded iron formations) then the overall environment would have to have remained anaerobic. This requirement carries with it the further corollary that the early photosynthesisers must have been able to live, grow and generate more oxygen in these continued anaerobic conditions.

At first glance the fossil record seems to support this hypothesis. Primitive algal-like fossils have been reported in 3.4 Gyr-old rocks^{17,18}, and stromatolites, common throughout the later Precambrian, are now known to have existed at least as far back as 3 Gyr ago¹⁹. For most people these ancient fossil occurrences confirm the existence of prokaryotic cyanophytes, the blue-green algae, at these times. Because all modern blue-green algae, including those that form stromatolites, are capable of oxygen-generating photosynthesis it seemed reasonable to conclude from the fossil record that this process was already in operation in the early Precambrian. Cloud⁹⁻¹¹ has gone one step further by concluding that if the later banded iron formations are intimately related to photosynthetic oxygen then their very presence in the oldest known sediments, the 3.8 Gyr-old Isua rocks of Greenland¹, suggests the existence of O₂-photosynthesis that long ago.

On the other hand, some workers^{16,20,21} have cautioned against such blanket assumptions pointing out that at least some of the early stromatolitic structures might have been the work of photosynthetic flexibacteria using a type of photosynthesis which does not release oxygen. Although it has been known for some time that blue-green algae can survive in anaerobic environments²², it was always doubtful that growth could be maintained indefinitely or, more importantly, that oxygenic photosynthesis was ever completely inhibited²³. Thus, the idea that blue-green algae (and their fossil counterparts) need not indicate oxygen-generating photosynthesis has had few supporters and little firm basis in fact. But recent biochemical and physiological work indicates that this idea should be reconsidered.

Oxygen photosynthesis

Oxygen photosynthesis consists of two basic photochemical pathways that normally operate in series: photosystem I and photosystem II. In photosystem II the water molecules split to donate electrons and release free oxygen. Photosystem I does not involve the release of oxygen and it is functionally homologous to the bacterial photosynthetic pathways. In the bacterial photosynthetic reactions hydrogen sulphide (or other agents²⁴) donate the electrons releasing elemental sulphur. The two generalised photosynthetic reactions are:



Recently, it has been demonstrated that, quite remarkably, several blue-green algae can grow, indeed thrive, solely on the anoxygenic photosystem I (refs 25, 26). In these carefully controlled experiments photosystem I operated independently of, and without needing photosystem II, photoassimilating CO₂ and using H₂S as the electron donor. With photosystem II shut

down water is not an electron donor and no oxygen is generated. As long as reducing conditions are maintained the photosystem II reaction is not required and growth continues. When oxidising conditions are re-established photosystem II is reactivated.

Cohen *et al.*²⁵ first demonstrated this remarkable activity in the blue-green alga *Oscillatoria limnetica*. Subsequent work by Garlick *et al.*²⁶ has shown that this capability exists in 11 of 21 strains examined, including both filamentous and unicellular blue-green algae from both marine and freshwater habitats.

More significantly, Oren *et al.*²⁷ have studied the comparative quantum efficiency of the two processes. In the reducing conditions photosynthesis was maintained by photosystem I at all wavelengths of visible light, including those wavelengths most favourable for photosystem II. Even in the low efficiency regions of photosystem I light is better utilised than in the low efficiency regions of photosystem II. Hence, Oren *et al.* suggested that "The patterns of photosynthesis in these blue-green algae would thus seem more oriented towards anoxygenic rather than oxygenic photosynthesis" and they concluded by saying that "this phototrophic pattern... represents metabolic relics of cyanobacterial evolutionary history."

Newly constructed evolutionary trees derived from biochemical data are also relevant. Analysing protein and nucleic acid sequences from a wide variety of prokaryotes and eukaryotes Schwartz and Dayhoff²⁸ proposed that blue-green algal cytochrome *c*₆, which functions in connecting photosystems I and II, is phylogenetically distant from the *c*-type cytochromes of the anaerobic photosynthetic bacteria. From their composite evolutionary tree they concluded that not only is photosystem I evolutionarily primitive, but more importantly, even aerobic respiration probably preceded oxygen-releasing photosynthesis and photosystem II.

Implications

What are some of the implications of these studies for the interpretation of the Precambrian environment and for the evolutionary history of the prokaryotic way of life? It is generally accepted that the first organisms were simple anaerobic heterotrophs²⁹ or possibly chemolithotrophs³⁰ which depended for their existence on the free energy available in whatever substances were present in the environment. As these primeval energy-rich substances became less plentiful and useless waste products accumulated, there would have been increased selection pressures on all forms of life to develop alternative energy sources. Light was the obvious, if not the only viable choice³¹. Thus, dwindling heterotrophic resources would have set the stage quite early for the evolution of the anaerobic photosynthetic bacteria and ultimately photosystem I—an ideal situation for organisms living in a Precambrian anaerobic reducing environment. The ancient anaerobic ecological system would have recycled organic material³² by means of fermentative breakdown reversing the photosynthetic reaction of equation (1) to produce H₂S and CO₂. The addition of volcanic gases containing hydrogen, carbon and sulphur components would have assisted in maintaining this reducing environment.

In such conditions, what selection pressures would there have been to develop and add in series the now dominant photosystem II? Obviously, water is a virtually unlimited and readily available resource as an electron donor but splitting the water molecule is thermodynamically costly, requiring more than six times as much free energy as the analogous H₂S reaction³¹. The data of Oren *et al.*²⁷ make it clear that with regard to quantum efficiency photosystem I is quite effective, taking good advantage of the entire visible spectrum. Furthermore, bacterial photosynthesis utilises bacteriochlorophyll rather than the chlorophyll of oxygen-generating photosynthesis. Once evolved, chlorophyll has a free energy advantage³³ but the biosynthesis of chlorophyll *a* requires molecular oxygen in one of its steps³⁴. Therefore, unless this aerobic step is a later modification the evolutionary develop-

ment of chlorophyll *a* in a Precambrian anaerobic environment would be unfavourable.

It, therefore, seems that although green plant photosynthesis releases oxygen to the environment it requires an oxygenic environment to get going in the first place. Molecular oxygen, then, 'primes the pump' for the further production of oxygen by photosynthesis. It is improbable that true oxygen-generating photosynthesisers could have evolved in the anaerobic conditions of the early Precambrian without an impetus provided by some other source of free oxygen. Somehow general aerobic conditions would have had to precede and thus stimulate the later evolutionary development of photosystem II.

These facts make it very unlikely that photosystem II evolved simply by chance in some early photoautotroph already well-adapted to the anoxygenic reducing environment. Only by removal of reduced substances as potential electron donors for photosystem I would there have been significant pressure on Precambrian prokaryotes to find alternate and more expensive electron donors and thus develop photosystem II. But elimination of reducing conditions implies the development of general oxidising conditions and this, in turn, requires a continuing source of free oxygen to remove not only the hydrogen sulphides but also to scavenge and oxidise the massive amounts of ferrous iron now tied up in the banded iron formations. Therefore, we must look to somewhere other than photosynthesis for the source of this free oxygen.

Oxygen-mediating enzymes

Additionally, proponents of photosynthesis as the major source of oxygen for the early banded iron formations have suggested as part of the biotic model that the primitive Precambrian anaerobes would probably not have had sufficient oxygen-mediating enzyme protection and that therefore they required the ferrous iron, readily available in their environment, as an O_2 -acceptor³⁻¹¹. Schopf¹⁶ has pointed out that it is difficult to visualise how extracellular ferrous iron could protect an intracellular biochemistry from auto-oxidative toxicity when the oxygen source is itself intracellular. However, many modern prokaryotes, including the photosynthetic bacteria do have oxygen-mediating enzymes that are clearly not later developments or secondary adaptations to our modern environment.

The first of these enzymes would include the superoxide dismutases. These enzymes function to protect against superoxide free radical toxicity. They had been thought to occur only in aerobic organisms but recently they have also been observed in various primitive obligate anaerobes³⁵⁻³⁸. Here their principal function is clearly to protect against temporary oxidising conditions. Lumsden and Hall³⁹ have suggested that the early evolutionary development of superoxide dismutase was in response to pre-photosynthetic photodissociated oxygen.

The second of these enzymes is the principal one used in all photosynthetic carbon fixation involving the Calvin cycle. Called ribulose-1,5-bisphosphate (RuP_2) carboxylase/oxygenase (formerly carboxydismutase), it catalyses the initial step of the Calvin cycle wherein carbon dioxide reacts with RuP_2 to form 3-phosphoglycerate (PGA). The relevant point here is that this cardinal enzyme of photosynthesis also reacts with molecular oxygen⁴⁰. Furthermore, oxygen and carbon dioxide compete with one another in a tightly coupled fashion⁴¹ for the identical active site on the enzyme⁴². The competitive aspect of this reaction can markedly affect net photosynthesis and this process, known as photorespiration, has been described as puzzling, inefficient and wasteful^{43,44}, especially in today's environment rich in oxygen and poor in CO_2 . But as Smith⁴⁵ has pointed out, this difficulty would not have been a problem in Precambrian conditions poor in O_2 and perhaps richer in CO_2 . The primitive nature of this enzyme is demonstrated by the fact that it occurs not just in the green plants but also in some anaerobic photosynthetic bacteria⁴⁶ where its oxygenase activity has been confirmed by *in vitro* experiments⁴⁷.

Discussion

What is the explanation for the presence and oxygenase activity of such an enzyme in anaerobic photosynthetic organisms and why has it not suffered extinction, at least in some of the higher plants? It seems plausible that in early Precambrian conditions the competitive reaction was not the biochemically wasteful, inefficient process it often is today. On the contrary, the ability to react with oxygen was most probably a built-in protective device for an anaerobic way of life where occasional transient oxygen could be lethal. In such conditions oxygen would have been detoxified through the formation of glycolate or CO_2 , either of which may have been reassimilated or released into the environment. It probably still has this role in modern anaerobic photosynthetic bacteria. It has survived extinction in the higher plants only because of its pivotal position in a critical metabolic cycle⁴⁸. It seems likely then that when photosynthesis first evolved protection against transient oxygen toxicity was needed. Ferrous iron would, of course, have been required as a general environmental oxygen sink maintaining the surrounding anoxygenic conditions but it would not have been necessary as an enzyme substitute. The much later development of the C_4 pathway of carbon fixation and of Kranz anatomy in higher plants⁴⁵ illustrate the complicated adaptive efforts that were necessary to circumvent the higher oxygenase activity required by a Phanerozoic oxygen-rich atmosphere.

The occurrence of both superoxide dismutases and RuP_2 -oxygenase in obligate anaerobes phylogenetically below the oxygen-releasing photosynthesisers eliminates any need for ferrous iron as a directly protective oxygen-acceptor for the early microbiotas.

The foregoing biological-biochemical arguments strongly support the view that atmospheric photodissociation rather than photosynthesis was the primary contributor of free oxygen to the earlier Precambrian environments. But does the geological-geochemical evidence also support this conclusion? New geological-geochemical data and reappraisals of existing data are placing increased pressure on the biotic model and its consequences.

The basic tenet of the biotic model that ferrous iron be in direct balance with photosynthetic oxygen presents, in addition to the biological difficulties discussed above, several inconsistencies with the geological and palaeontological evidence. In the conditions imposed by this model the precipitated ferric iron should be closely associated with some evidence of the microorganisms it was presumably protecting. This is especially true because the oxygen used to oxidise the iron could not also be used to oxidise the organic carbon of dead phytoplankton. But generally in the banded iron formations, organic carbon is inversely correlated with ferric iron regardless of facies, being especially low in the hematitic facies⁴⁹. The early stromatolites are seldom directly associated with ferruginous encrustations^{16,50}, being primarily calcareous or siliceous instead. For example, although there are ferruginous mudstones in facies of the 3 Gyr-old Pongola Supergroup in South Africa, the stromatolites in these sediments are themselves calcareous⁵¹. The problematical microfossils described by LaBerge⁵² in banded iron formations are associated with the silica deposition rather than with the precipitation of iron. For the banded iron formations it could be argued that some physical process sorted and transported the dead phytoplankton to some other burial site. But then for the stromatolites the opposite process is required, transporting the precipitated iron, sorted from the carbonate, to some other resting place. Furthermore, the vast quantity of buried organic carbon needed to balance the oxygen used in precipitating this iron has yet to be found⁵³.

There are additional problems. If the early photosynthetic oxygen is in direct balance with ferrous iron then there should be no evidence of terrestrial oxidation of iron because the oxygen required should not have escaped into the atmosphere. Recent discoveries also apparently contradict these predictions of the biotic model. Dimroth and Kimberley⁵⁰ report on red

beds, oxidised weathering crusts and hematite coatings on detrital grains in Canadian deposits "well beneath one of the largest cherty iron formations in the world." Förster and Wachendorf⁵⁴ have reported quartzites with relics of original hematite crusts in the 2.3 Gyr-old stromatolitic Transvaal Dolomite of South Africa and they too interpret these as documenting an oxidative weathering at that time.

The biotic model calls for the general absence of sulphate deposits until the latest Precambrian (~800 Myr) and reports of major evaporite deposits from the lower Proterozoic of Canada⁵⁵ and from the mid-Proterozoic of Australia⁵⁶ also seems to contradict this requirement.

The criteria from geochemical fossils, such as chlorophyll derivatives or light-reduced carbon isotopes found in early Precambrian rocks are, unfortunately, of little help. They might be used cautiously to delimit the onset of photosynthesis *per se* but not, at present, for the appearance of oxygen-generating photosynthesis. This is because the fossil porphyrins could have been derived from either bacteriochlorophyll or from chlorophyll. The isotope data, even if primary and unaltered, indicate carbon isotope fractionation and CO₂ fixation by the RuP₂ carboxylase enzyme which, as described above, occurs in both types of photosynthesis and with similar results⁴¹.

Conclusions

The accumulated evidence offers less support for a photosynthetic origin for the early Precambrian oxygen. However, the alternative abiotic photodissociation model is not without its own consequences and difficulties. These include the presence of stream deposited uraninites and the comparative absence of terrestrial red beds, both of which seem anomalous had the Precambrian atmosphere been generally oxidising^{9,10}. It has also been emphasised that the movement of reduced iron in solution throughout the surface waters of the Precambrian seas would have been inhibited by a generally oxidising atmosphere. Here the questions of the concentration or partial pressure of oxygen and effectiveness and stability of the photodissociation mechanisms become important. There has been much speculation over these points and very little agreement^{12,14,15,57}, partially because of the interpretation of data on the present atmosphere but principally because of the varied possibilities that exist for the composition and structure of the early atmosphere^{58,59}. Simpson and Bowles⁶⁰ have argued that the preservation of uraninite need not have required a reducing atmosphere, offering as evidence the detrital uraninites of the present-day Indus River of Pakistan. The most recent geochemical work on the precipitation of hydrous ferric oxides⁶¹ and on the dissolution-oxidation of uraninite⁶² suggests that hydrospheric pO_2 values of $> \sim 10^{-13}$ but $< \sim 10^{-3}$ would be reasonable. The atmospheric oxygen concentration of about 10^{-4} , suggested by Berkner and Marshall¹⁴ to have resulted from photodissociation, falls within this range.

The fact that the iron formations are so commonly banded implies a cyclic or periodic instability in the availability of oxygen or ferrous iron or both. Hydrospheric instability (upwelling or seasonal overturn) is an explanation consistent with banding⁶³⁻⁶⁵. But could there also be some sort of cyclic atmospheric instability acting either in combination with, or independently of, hydrospheric instabilities? Even if no data are now consistent with such cyclic atmospheric instability this suggestion is worth considering. First, periods of oxygenation could now punctuate the general reducing conditions in both the terrestrial and the marine environments. The ferrous iron sink would ensure that the general environment remained anoxygenic. Thus, limited evidence of red beds or sulphates would no longer be inconsistent with occurrences of detrital uraninites as long as the peak levels of pO_2 at the uraninite localities did not substantially exceed the Berkner-Marshall level of 2×10^{-4} atm, or 0.001 PAL (present atmospheric level)⁶². A very early Precambrian (3.8 Gyr) origin for photosystem II (O₂-generating photosynthesis) is not required to explain the Isua banded iron formations of Greenland⁹⁻¹¹

and no simultaneous burial of organic carbon in balance with the oxygen of the iron formations is necessary. The so-called missing carbon problem is thus abated. The stromatolites or other algal fossils need not be encrusted with iron oxides, their siliceous or calcareous composition making better sense as organisms lacking the ability to generate oxygen.

Eventually, however, the general inorganic sinks for photolytic oxygen would begin to be exhausted, in local basins at first but then on a world-wide scale. The appearance of extensive rather than local oxidised deposits of iron and sulphur (red beds and evaporites) should signal the beginning of the end of the general Precambrian reducing hydrosphere. Present evidence suggests that this may have taken place in an interval ~1.8–2 Gyr-ago^{11,16}. At this time, the banded iron formations reached their culmination⁶⁶. Extensive sulphate deposits occur⁵⁵, and older subaerial red beds are now being found^{11,50}. The oxidation state of some rare earth elements changes after this time⁶⁷. The gradual elimination of more and more of the reducing environments, and therefore of sulphides as potential electron donors, would have immediately placed the requisite evolutionary pressure on the local microbiotas to seek alternative reductants and develop aerobic respiration and the more expensive water-splitting and oxygen-generating photosystem II. The biotic formation of free oxygen could then begin to supplement and eventually overtake the abiotic photodissociative sources eliminating the world-wide anoxygenic environment and initiating the build-up of the oxygenic atmosphere as we know it. In this transition period where conditions might rapidly change from oxidising to reducing and back again, those blue-green algae capable of facultative anoxygenic photosynthesis alternating between photosystem I and photosystem II²⁵ were likely to have been the reigning life forms in near-surface waters, taking advantage of either situation but ready for the oxygenic world that they themselves were destined to widen. The absence, to date, of major coeval deposits of reduced carbon indicates that this process was slow and not of the type envisaged by Berkner and Marshall¹⁴. The build-up of oxygen in the atmosphere to near present levels did not take place immediately. But from that time one of the dominant strategies of life was to adapt to increasingly oxygenic conditions.

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Dinosaur feeding behaviour and the origin of flowering plants

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A reappraisal of current theories suggests that the origin of flowering plants was closely correlated with the spread of big, low-browsing dinosaurs.

DARWIN called the origin of flowering plants an 'abominable mystery' because angiosperms seemed to appear abruptly, fully developed, in Cretaceous rocks without a clear hint of their immediate ancestry¹. Analysis of terrestrial community structure during the Jurassic-Cretaceous transition, presented here, strongly suggests that a swift and extensive change in the feeding behaviour of herbivorous dinosaurs was one key ecological event which opened opportunities for the rapid initial diversification of angiosperms.

Time and place of angiosperm origins

Undoubted angiosperm leaves appear first in late Early Cretaceous sediments^{2,3}. Because many specimens were referred initially to extant genera and families, it seemed that a surprising variety of modern types was already present. According to the theory championed most vigorously and eloquently by Axelrod^{4,5}, flowering plants evolved long before the Cretaceous, perhaps as early as the Permian, but were restricted to certain habitats, such as the well-drained granitic highlands of the tropics, which were distant from any low-lying depositional basin where fossils might be preserved. According to this view, the mid-Cretaceous debut in the fossil record does not mark the evolutionary origin of angiosperms but rather their dispersal down into the coastal lowlands where sediments and fossils were forming. Recent developments in plate tectonics force reappraisal of part of this argument. Some plant-bearing deposits, now located along continental coastlines, were originally formed deep in continental interiors. The Newark Series (Mid-Late Triassic-Early Jurassic) of the American North Atlantic coast was deposited in rift valleys adjacent to granitic highlands with rugged local relief, deep in the interior of the Laurasian supercontinent; later this supercontinent was pulled apart by a spreading axis lying under the rifts⁶. The Newark basins seem ideal for trapping remains of the postulated pre-Cretaceous angiosperms, for palaeolatitudes were as low as about 20°N and the facies (red beds and nonmarine evaporites) indicate a hot, seasonally arid climate and well-drained soils^{7,8}. But no trace of angiosperms has been found among the Newark pollen and leaf floras.

Barghoorn, Doyle & Hickey, and Hughes^{2,9,10,11} believe that the Cretaceous leaf and pollen record does not show the alleged simultaneous entry of many modern types, but rather begins with a few structurally primitive leaf and pollen forms. Most modern families are not clearly identifiable until the Eocene or later. These authors make a persuasive case that the

angiosperm fossil record can be read literally as showing a gradual increase in diversity and complexity during the Cretaceous and Early Tertiary without the need to postulate a long unrecorded pre-Cretaceous history.

It has been argued that the earliest angiosperms were large trees in tropical climax forests, because many primitive living species have this growth habit¹². However, recent work on the functional morphology and taxonomy of Cretaceous angiosperms strongly suggests that during the initial adaptive radiation most species were woody shrubs in disturbed habitats. Doyle & Hickey argue that carpel closure, reduction of the male gametophyte and polyploid endosperm appeared as adaptations to compress the life cycle and thus increase the capacity to colonise habitats which were frequently disturbed by drought, erosion or sedimentation^{3,9,10}. According to Hickey the diagnostic features of the earliest angiosperm leaves—small size, simple margins, poorly defined petiole and poorly differentiated ranks of venation—were acquired during evolutionary reduction of a large compound leaf in a severely semiarid environment. Early angiosperm leaves often dominate the fossil macrofloras in the mid-Cretaceous (Late Albian-Cenomanian) localities, and most of these sites represent river channel and levee deposits; environments where rapid erosion and deposition would inhibit the development of a climax forest. In sharp contrast, most of the mid-Cretaceous microfloras come from fine-grained lacustrine and near-shore marine deposits, environments which received wind-blown and water-blown pollen from the stable forests along lakeshores and on the coastal flatlands^{13,14}. In these pollenfloras conifers, not angiosperms, dominate. Two unusual North American mid-Cretaceous pollen localities do show abundant angiosperms—a mottled, flood-plain facies in the Dakota Group of Minnesota¹⁴ and a local swampy facies in the Arundel Formation of Maryland¹⁵. Significantly, both of these depositional environments represent rapidly shifting physical conditions.

Jurassic dinosaur browsing behaviour

Major events in the evolution of terrestrial vertebrate herbivores are often ascribed to the influence of evolutionary floral changes—the spread of grasslands in the mid-Tertiary is generally cited as the ultimate cause of much extinction and adaptive radiation among mammals. But the selective pressure in plant-herbivore coevolution can work in the opposite direction—large herbivorous tetrapods can be a powerful agent in plant evolution. The single most important aspect of browsing pressure is height: high-browsers (for example, giraffes) depend on the long-term production of leaves, fruit and buds from trees and rarely cause the death of the adult sporophyte;

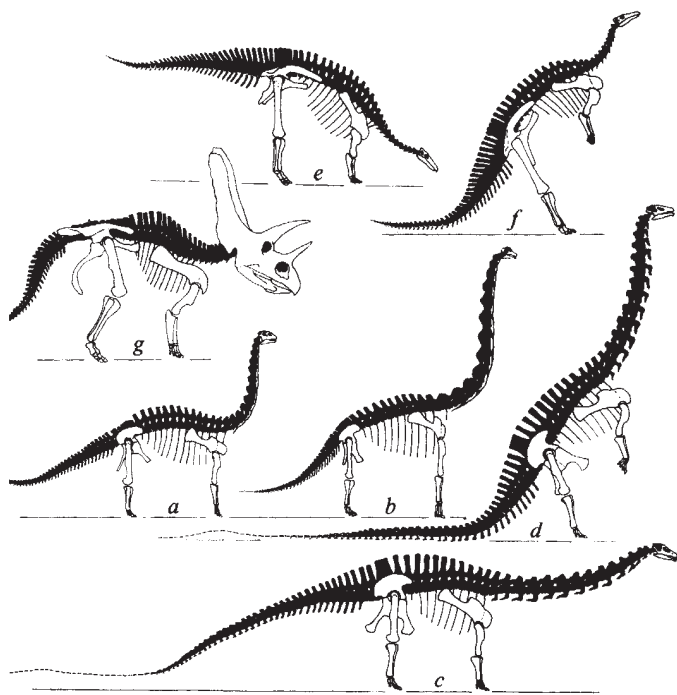


Fig. 1 Low-browsing and high-browsing adaptations in large herbivorous dinosaurs. All skeletons are drawn to the same shoulder-to-hip length. *a*, Primitive cetiosaurid sauropod (*Haplocanthosaurus*): long trunk, long forelimbs, relatively small pelvis and tail; *b*, brachiosaurid sauropod (*Brachiosaurus*): basic cetiosaurid plan with exceptionally long neck; *c*, advanced diplodocid sauropod (*Apatosaurus*): shortened trunk, shortened forelimbs, relatively huge pelvis and tail; *d*, diplodocid in tripodal feeding stance, weight supported by enlarged hindlimbs and tail; *e*, stegosaur: shortened forelimbs, relatively huge tail; *f*, stegosaur in tripodal stance; *g*, fully quadrupedal ornithischian (ceratopsian *Pentaceratops*): relatively small tail.

low-browsers concentrate on the production of annuals and seedlings of larger plants, and the supply of low-browse depends in part on the continuous regeneration and recolonisation by shrubs and seedlings. During the first 100 Myr of land vertebrate herbivore history, from the Late Carboniferous until the early Late Triassic, all the big herbivores were short-necked, short-limbed, low-browsers (diadectids, edaphosaurs, deinocephalians, pareiasaurs, dicynodonts, rhynchosaurs, gomphodonts and aetosaurs). But in the mid-Late Triassic this trophic role was taken over by prosauropod dinosaurs—long necked forms with long hindlimbs, powerful pelvis and tail. Prosauropods probably could feed tripodally—supporting their weight on the hindlimbs and stout tail, much as modern varanid lizards do when threatened¹⁶. This shift from low browsing to high browsing among the big herbivores must have changed the selective pressure on the browse plants; in Gondwanaland a major evolutionary floral shift did occur shortly after the prosauropods became common: the *Dicroidium* complex, a diverse group of seed ferns characteristic of the Triassic, became nearly entirely extinct and was replaced by a Jurassic radiation of conifers.¹⁷

By mid-Jurassic, sauropods had replaced prosauropods. Interpretation of Jurassic terrestrial community evolution has been hindered by the reconstruction of sauropods as aquatic hippopotamus-like browsers. Comprehensive review of sauropod anatomy has shown that sauropods basically were terrestrial^{18,19}. Field analysis of the facies-distribution of dinosaurs in the Morrison Formation, which has produced the most abundant and diverse sauropod sample, confirms the terrestrial interpretation. Sauropods are the dominant big herbivores in nearly all environments, including oxidised flood plain sediments which do not contain the remains of undoubtedly aquatic vertebrates (crocodilians, turtles and fish)²⁰. In

sharp contrast, in the Plio-Pleistocene of East Africa, the various species of hippo are most common in river channel and lake margin sediments, where crocodiles are common; hippos and crocodiles decrease markedly in frequency in flood plain sediment where terrestrial groups, bovids, giraffids and so on, are dominant²¹. Speculation²² that sauropods could not have been terrestrial high browsers because of the problems of pumping blood to the head is, I believe, a misapplication of uniformitarianism. It would be difficult to 'design' a high-browsing mammal twice as tall as a giraffe if the heart alone had to pump blood to the head in one movement. But the sauropods could have used contractions of neck musculature as a relay pump to carry the cranial arterial supply. Somatic muscle contractions are important in venous return, and an arterial muscle relay pump for distal cervical and cranial regions would not be surprising. It is unnecessary to force a model of sauropod biology to be constrained by the assumptions of a single pump mechanism.

Sauropod communities show a diverse array of adaptations for feeding on various levels above the ground and on different plant tissues (Figs 1 and 2). The two most primitive sauropod families, the cetiosaurids and brachiosaurids, were quadrupedal with short neural spines over the hips, as in quadrupedal ornithischian dinosaurs, and relatively small tail and pelvis. Neck length and shoulder height determined browsing level in these animals—forelimbs and neck were of moderate length in cetiosaurids and extremely long in brachiosaurids (Fig. 2). Camarasaurids resembled cetiosaurids in general, but had more powerful tails, suggesting some tripodal feeding, and split cervical neural spines which would have increased the size and leverage of neck muscles for pulling leaves and branchlets off conifers. The most advanced sauropods, the diplodocids, were highly specialised for tripodal feeding; the back and forelimbs were very short, the neural spines over the hips were very tall, as in large bipedal dinosaurs, the pelvis and the base of the tail were extremely powerful, and the chevrons were sled-shaped to support the weight of the hindquarters.

Relatively small (50–500 kg) short-necked, low-browsing ornithopod dinosaurs, camptosaurus and hypsilophodonts, are present but usually rare in Jurassic localities. The most conspicuous ornithischians were the stegosaurs (2–4 tons) which paralleled diplodocids in the tripodal adaptations of short forelimbs, tall sacral neural spines and powerful transverse processes of the anterior caudal vertebrae (Fig. 1). In nearly all Mid-Jurassic and Late Jurassic sites the high-browsing sauropods and stegosaurs make up 95% or more of the preserved biomass²³.

Fig. 2 Late Jurassic (lower) and Late Cretaceous (upper) large herbivores compared. Figures represent fully adult individuals shown to scale. In the upper figures each ornithischian is shown browsing close to the ground and also with the maximum vertical reach permitted by the limbs and vertebral articulations. Morrison Fauna (lower): *a*, *Haplocanthosaurus*; *b*, *Brachiosaurus*; *c*, *Camarasaurus*; *d*, *Barosaurus*; *e*, *Diplodocus*; *f*, *Apatosaurus*; *g*, *Stegosaurus*; *h*, *Camptosaurus* (the largest Morrison low-brower). Kirtland-Fruitland-Ajo Alamo Fauna (upper): *a*, *Alamosaurus*; *b*, *Parasaurolophus*; *c*, *Pentaceratops*; *d*, ankylosaur. The vertical scale is given in metres.



The diversity of cranial and dental adaptations indicates that the Jurassic high browsers took a wide range of conifer tissue. Camarasaurid teeth were stout, implanted vertically in relatively short, strong jaws, show severe tooth-to-tooth and tooth-to-food wear, and must have been used to crop-resistant plant parts. Diplodocids had elongated tapering snouts with delicate, procumbent teeth for selecting smaller plant parts. The posterior position of the nostrils in diplodocids, often cited as an aquatic adaptation, may well have functioned to protect the external nares as the head was thrust into the conifer canopies. Stegosaurids had very long rostra with a small, terminal beak; brachiosaurid teeth were intermediate between those of camarasaurids and diplodocids.

Cretaceous browsing behaviour

Cycads and conifers would have been relatively slow to recolonise bared ground. The sauropod-stegosaur communities probably cropped leaves, branchlets and cones without causing serious mortality among the adult trees, although some seedlings must have been destroyed by young sauropods, diplodocids and stegosaurs feeding quadrupedally, and by smaller, low-browsing ornithomimids. At the Jurassic-Cretaceous boundary the herbivore communities changed considerably. Stegosaurids and most of the sauropod lineages became extinct or were greatly reduced in numbers and diversity. The earliest Cretaceous faunas from England and Germany (Wealden Facies) contain new groups of big, low-browsing ornithischian dinosaurs (Fig. 3): the bipedal iquanosaurids, dome-headed pachycephalosaurs, and the armoured nodosaurids²⁴⁻²⁸. The postcranial adaptations of all of the Cretaceous ornithischians would have restricted their browsing to very close to the ground. The vertebral column had a strong downward and forward curvature in the thoracic region; this flexure was permanent, fixed by the orientation of the neural spines and zygapophyses. An analogous flexure occurs in some living low-browsing bovids, such as *Bison*. The base of the tail in Cretaceous ornithischians was usually weak compared with that of stegosaurs; tripodal posture was not well developed. During the Mid-Cretaceous and Late Cretaceous more groups of big ornithischians were added to the fauna (Fig. 3): parrot-beaked psittacosaurids, protoceratopsids, ceratopsids, ankylosaurids and the duck-bills (hadrosaurs).

Partitioning of the plant resources was complex—ankylosaurs and hadrosaurs had short, square beaks for browsing very close to the ground; ankylosaurs had weak teeth and were restricted to low-fibre food; hadrosaurs had powerful shearing teeth for high-fibre food; horned dinosaurs had long rostra and pointed beaks for browsing among low-growing shrubs and very strong, slicing teeth for cutting the toughest of plant material.

The extinction of Jurassic high browsers and the rapid diversification of big Cretaceous low browsers changed the selective pressure on the plant communities. Intense low browsing would have increased mortality among seedlings, thinned out the forest structure, and would have favoured shrubs which could maintain a dynamic steady-state with the herbivores, rapidly regenerating the cropped foliage and colonising areas laid bare by close cropping. Angiosperms appear first in the Late Neocomian, some 5–10 Myr after the beginning of the Cretaceous ornithischian radiation (Fig. 3). Angiosperm abundance and diversity builds gradually through the succeeding Aptian and Albian, and by the middle of the Late Cretaceous angiosperm leaves and pollen gain dominance over conifers in many samples. Thus the angiosperm radiation seems to be an evolutionary response to the change from high to low browsing at the Jurassic-Cretaceous boundary. The angiosperm adaptations for compressed life-cycle and rapid recolonisation would be favoured by the intense cropping of the Cretaceous ornithischians. The number of ornithischian families continues to grow through the last half of the Cretaceous as the number of angiosperm groups increases.

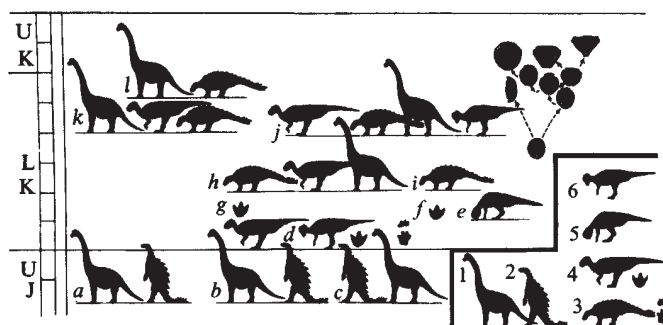


Fig. 3 Stratigraphic record of dinosaur herbivores and the origin of flowering plants. The dinosaur symbols stand on a line indicating the stratigraphic position of the fauna. Faunas: a, Morrison; b, West European Kimmeridgian; c, Tendaguru (East African); d, Mid-German Wealden; e, Chinese-Mongolian psittacosaur localities; f, Ashdown Sands, English Wealden; g, Ataplia site (Kazakhstan); h, Wadhurst Clay, English Wealden; i, Kampingay locality (Russia); j, Upper Wealden, England; k, Cloverly-Cedar Mountain (western North America); l, Arundel (eastern North America). 1, Sauropod; 2, stegosaur; 3, ankylosaur (footprints indicated); 4, large ornithomimid (footprint indicated); 5, psittacosaur; 6, pachycephalosaur (dome-head). Pollen symbols show the first appearance of angiosperms in the Barremian stage and the successive diversification. LK, Lower Cretaceous; UK, Upper Cretaceous; UJ, Upper Jurassic. Data from refs 3, 15, 23–29.

In summary, sauropod and stegosaur dinosaurs coevolved with conifer-dominated forests during the last half of the Jurassic; sudden extinction of most of the sauropod-stegosaur complex was followed by rapid evolution of big, low-browsing ornithischians; within a few million years after this shift to low browsing, the angiosperm radiation began; the primitive angiosperms were probably low shrubs and small trees which had a distinct advantage over conifers and cycads in areas subjected to intense low cropping. Once acquired, the life-history strategy of angiosperms gave them a competitive advantage in many habitats, even those lacking strong browsing pressure. Many evolutionary breakthroughs are induced by a sudden change in the environment. The initial development of flowering plants probably was, in part, a direct result of changes in dinosaur feeding behaviour.

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letters to nature

Two X-ray periodicities from the vicinity of 4U1145-61

EXTENDED Ariel V observations in September and December 1977 centred on 4U1145-61 reveal two X-ray periodicities at 4.95 and 4.86 min. The amplitude of both varied, the former by a factor 2, the latter by a factor 7. The shorter period decreased by 0.0015 min over an interval of 50 d while the longer increased by 0.0034 min in the same period. Measurements of the pulse phase revealed no evidence for orbital motion and upper limits to $a_x \sin i$ are given. It is not possible to determine whether these modulations both originate from 4U1145-61 or if there is an unreported source within 1° of 4U1145-61. The X-ray spectrum was unusual with a break at ~ 9 keV. We have obtained spectra of the optical counterpart, HEN715, at $25 \text{ \AA mm}^{-1}$ resolution in the range 3,700–5,300 $\text{\AA}$ and 6,200–7,200 $\text{\AA}$. The $H\alpha$ emission line was observed to change significantly in shape and central wavelength on a time scale of ~ 30 min. We set an upper limit to pulsations in the $H\alpha$ line of 5% relative to the continuum.

The Ariel V 2–15 keV proportional counter spectrometer¹ (PCS) observed the X-ray source 4U1145-61 for two periods of 14 d in 1977, beginning on 20 September and 30 November. Each two days of data taken with either 64s or 24s time resolution were subjected to periodogram analysis². Two sets of significant peaks were observed in the periodograms corresponding to periodicities at 4.86 and 4.95 min. The two modulations were detected simultaneously with both the time resolutions available. Four periodograms are shown in Fig. 1 illustrating that the relative amplitude of the two periodicities varied with time. The 4.86 min modulation was weak throughout the first observation (although it was still detected significantly, with probabilities that it could be caused by random processes of $<10^{-4}$). During the second observation this peak dominates the periodogram, an increase in amplitude of about a factor of 7. The amplitude of the 4.95 min periodicity varied by a factor 2 on a time scale of days during the first observing interval; it was relatively constant during the second (compare Fig. 1). It is not possible to determine the pulse fractions precisely because the background signal contributed by nearby strong sources (usually either 3U1223-62 or Cen X-3) is unknown. However, the maximum pulse fraction seen for the 4.86 min modulation during the second observation was $>50\%$. Both modulations were sinusoid-like and there was no evidence from the periodograms for any power at the first harmonics of the fundamental frequencies.

The phase of the two modulations during each 6 h block of data was determined by simultaneously fitting two sine functions with periods equal to the mean derived from the periodograms. The accuracy to which the pulse phase could be measured was limited by a ± 8 s uncertainty in the epoch of the data from each satellite orbit. The resulting phase measurements of both the 4.86 and 4.95 min periods for the two observations (four data sets) were then each fitted by a second order polynomial to derive the pulse period and its first derivative $\dot{P}$. These are listed in Table 1. A significant increase ($\dot{P}/P = +8 \times 10^{-3} \text{ yr}^{-1}$) was measured in the 4.86 min pulsations within the December observing interval. Comparing the periods derived for the September and December observing intervals as a whole we find that, contrary to the trend noted above, the 4.86 min period decreased by 0.0015 min ($\dot{P}/P = -2 \times 10^{-3} \text{ yr}^{-1}$). Over the same interval the 4.95 min period increased by 0.0034 min ($\dot{P}/P = +5 \times 10^{-3} \text{ yr}^{-1}$).

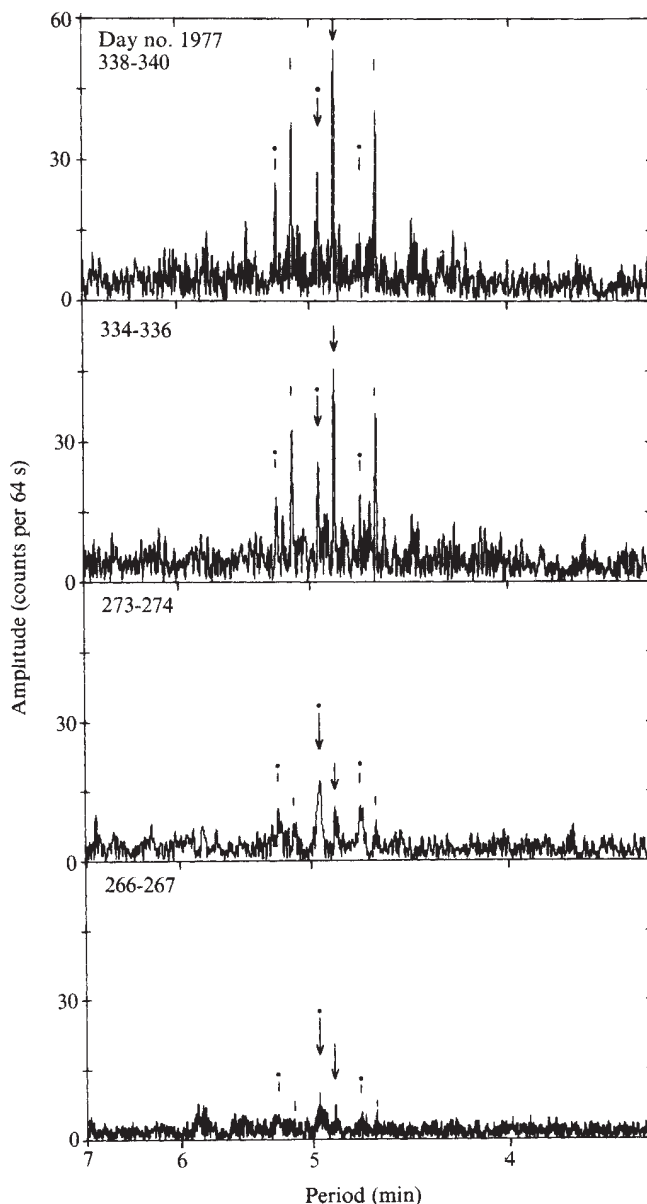


Fig. 1 Four periodograms of 4U1145-61. The data sets are not continuous but broken up every 100 min by Earth occultation of the source and other operational constraints. Thus any peak in the periodogram will be convolved with the window or sampling pattern (compare ref. 10). The central peaks are indicated by arrows, the predicted position of the first set of window peaks by bars. The excess of power at ~ 5.8 min seen on day 266-267 is from 3U1223-62 (compare ref. 2) which was for this part of the observation only within the 3.75° FWHM field of view.

Table 1 4U1145-61 pulse parameters

	Epoch (1977 day no.)	Pulse period (min)	$\dot{P}/P$ (rate of change of period per yr)
4.95 min	269.5	4.9549 ± 0.0002	$-(6.4 \pm 8.5) \times 10^{-3}$
	337.0	4.9583 ± 0.0003	$+(4.0 \pm 15.9) \times 10^{-3}$ *
4.86 min	269.5	4.8648 ± 0.0002	$-(2.7 \pm 8.0) \times 10^{-3}$
	340.5	4.8633 ± 0.0001	$+(8.0 \pm 2.7) \times 10^{-3}$

Errors are $\pm 3\sigma$.

* During this observation the satellite pointing position gradually drifted several degrees to the north of 4U1145-61 and it was not possible to track the phase of this period after 7 December 1977 (day 341).

To test for periodic phase shifts induced by orbital motion the second order term in the polynomial was replaced by a sine function. There was no evidence for such an effect in any of the four data sets and the two best upper limits to the projected semi-major axis, $a_x \sin i$ as a function of orbital period are displayed in Fig. 2 for both modulation periods. The preliminary report¹¹ of a 14-18 d periodicity was based on 'quick-look' data and was subsequently found to be spurious.

The PCS was operated in its spectral mode¹ for intervals of five hours every two days during the second observing period. No simple spectral model (power law, black-body or bremsstrahlung) gave an acceptable fit to the integrated spectrum. The data could, however, be represented by a simple continuum (for example, black body of temperature ~ 2 keV) with a large low energy excess or by a power law with a break at ~ 9 keV. The energy spectrum of the latter can be represented by a function of the form:

$$I(E) = \begin{cases} A \exp(-\sigma N_H) E^{-\alpha} & E < E_a \\ A \exp(-\sigma N_H) E^{-\alpha} \exp\left(\frac{E_a - E}{E_T}\right) & E > E_a \end{cases}$$

where E_a determines the position of the break (compare ref. 3). The best fit parameters for this function are given in Table 2 and illustrated in Fig. 3. Boldt *et al.*⁴ interpret a similar high energy break in the spectrum of Her X-1 in terms of modified

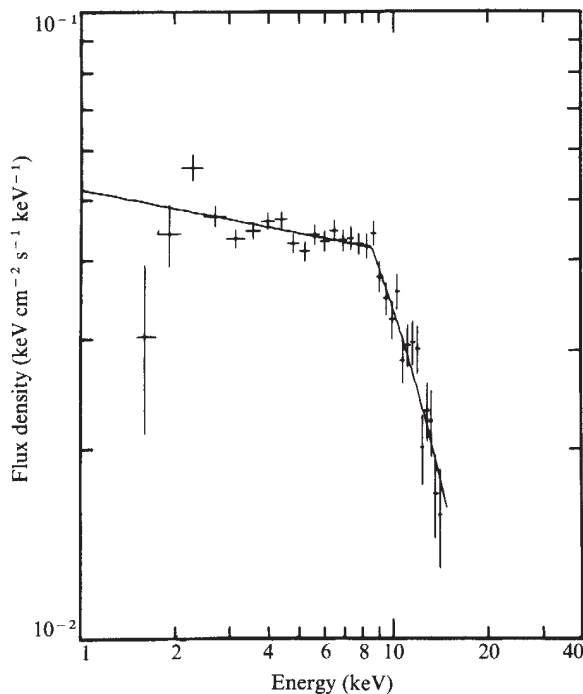


Fig. 2 2σ Upper limits to the projected semi major axis $a_x \sin i$ as a function of trial orbital period P_o for the two pulse periods. Three lines of constant mass function $(a_x \sin i)^3 / P_o^2$ are indicated.

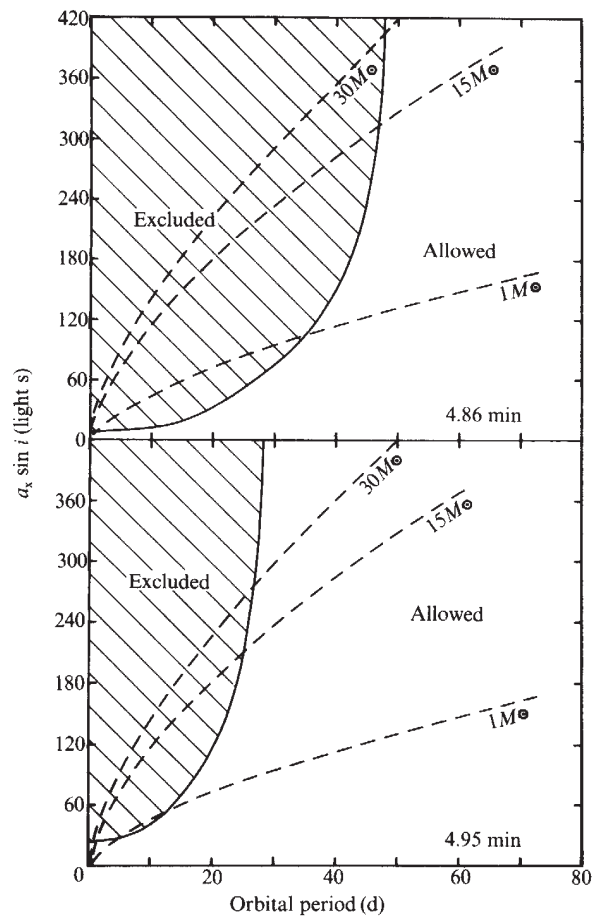


Fig. 3 The energy spectrum for 4U1145-61. The solid line is the best fit function given in Table 2.

Thomson scattering which dominates the radiative transfer in a highly magnetised plasma near the surface of a neutron star.

The 9th mag B emission line star HEN 715 lies within the ~ 30 arc s SAS 3 error circle for 4U1145-61 (ref. 5) and this identification is strengthened by our discovery of X-ray pulsations (compare refs 6, 7). Spectra of HEN 715 were obtained at a dispersion of $25 \text{ \AA mm}^{-1}$ in the range 3,700-5,300 $\text{\AA}$ and 6,200-7,200 $\text{\AA}$ during February 1977 using the image photon counting system⁸ attached to the 3.9 m Anglo-Australian telescope. The spectrum is dominated by emission at $H\alpha$ which has an equivalent width of 16 $\text{\AA}$ and a FWHM of $\sim 400 \text{ km s}^{-1}$. $H\beta$ and $H\gamma$ are seen in emission. The remainder of the Balmer series are present in absorption out to $\sim H_{12}$. The presence of the Balmer series together with prominent He I absorption lines supports an early B classification. The lines are all broad indicating a dwarf star (the star was classified as B1 Vne by Feast *et al.*⁹).

During one 25 min observation we accumulated individual spectra every 100 s in a 900 $\text{\AA}$ band centred on $H\alpha$, again at $25 \text{ \AA mm}^{-1}$ dispersion. We have used these data to search for an optical analogue of the X-ray periodicities. An upper limit of 5% can be imposed on periodic variability of $H\alpha$ relative to the local continuum (or vice versa) at a period of ~ 5 min. It was noted that in the 25 min interval between two observations of $H\alpha$, the profile and central wavelength of the line changed significantly (Fig. 4). No change was observed in the wavelength of the nearby atmospheric B-band which confirms that this is a real source effect.

Apart from the dual periodicities 4U1145-61 is similar in many respects to several other slowly pulsating X-ray sources such as X-Per, 3U1223-62, 3U1258-61 (compare ref. 6). The optical counterpart is an early B emission line star that has a probable mass of $\sim 15 M_\odot$. Consequently we see from Fig. 2

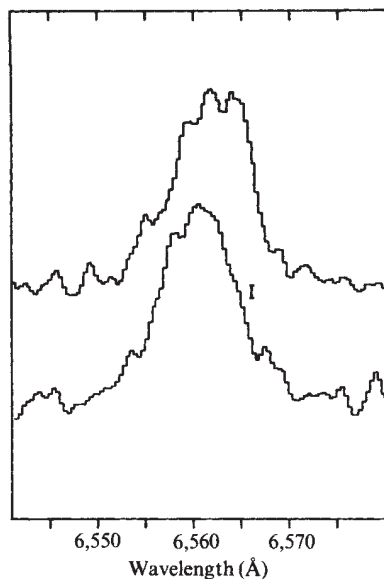


Fig. 4 The $H\alpha$ line from Hen 715. The 1σ uncertainty is indicated.

that the orbital period of the system is probably greater than ~ 45 d. The most likely distance for a star of this class is between 1 and 2 kpc which implies a mean X-ray luminosity of $\sim 10^{35}$ erg s $^{-1}$. Thus for the implied orbital separation of the system the rate of mass loss from HEN 715 required to power the X-ray source by accretion onto a neutron star via a stellar wind is between 10^{-5} and $10^{-7} M_{\odot}$ yr $^{-1}$, for wind velocities between ~ 500 and $1,000$ km s $^{-1}$.

Table 2 Spectrum of 4U1145-61 1977 day 334 to 347

$A = 0.052$
$\alpha = 0.09 \pm 0.12$
$N_H = < 5 \times 10^{21}$ hydrogen atoms cm $^{-2}$
E_a (keV) = 8.5 ± 0.7 keV
E_r (keV) = 7.3 ± 1.1 keV
$\chi^2 = 42$ for 26 degrees of freedom

It is difficult to account for the two periodicities separated by about 6 s using current models for X-ray pulsators, in which matter is funnelled onto the poles of a rotating neutron star. Both modulations are present when the data are examined in intervals as short as 0.25 d. One possibility which cannot be discounted is that we are observing two separate sources. By using the fact that the pointing direction of the spacecraft drifted on a time scale of days during these observations we find that both modulations must originate from within about 1° of 4U1145-61. There are no other catalogued sources in this area of sky. Thus because of the potential impact of this result on theories of pulsating X-ray sources it is important to demonstrate that both periods do originate from 4U1145-61, and to search for similar phenomena in other X-ray pulsars.

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Hydrogen $L\beta$ and $L\alpha$ emission lines observed from the interplanetary medium by the Voyager UV spectrometer

OBSERVATIONS of the interstellar medium in the 500-1,700 Å region of the optical spectrum reveals three measurable lines identified as He (584 Å), H Ly- β (1,025 Å), H Ly- α (1,216 Å). The origination of these emissions is dominated by resonance scattering of solar radiation by the interplanetary medium. Here we discuss the first measurements of the H Ly- β line by the Voyager ultraviolet spectrometers and our ability to detect sources other than local singly scattered radiation. The Voyager spectrometers¹ were designed primarily for observation of planetary atmospheric emission.

Resonance scattering of sunlight by neutral hydrogen and helium of the interplanetary medium gives rise to diffuse emission over the whole celestial sphere. The line of sight column density varies with direction because the Solar System is moving through the local interstellar medium and the distribution is perturbed by interaction with solar gravitation and radiation fields. The study of the interaction of the interstellar wind with the Solar System is reviewed in refs 2-4. Measurement of the resonance scattered emission from hydrogen and helium is an important technique in these studies.

Figure 1 shows a spectrum in the 500-1,700 Å region obtained from the UV spectrometer on Voyager 2 in interplanetary cruise. The spectrum is the summation of 696 12-min spectra obtained around day 263 1977 while pointed in a mean direction of $324^\circ \alpha$, $-23^\circ \delta$. The two prominent spectral features are the helium 584 Å line and the hydrogen Ly- α line at 1,216 Å. The feature at 1,025 Å is the hydrogen Ly- β emission line and represents the first measurement of this emission line from the interplanetary medium. Estimates of the intensities of the observed features with statistical error, in order of increasing wavelength, are 3.8 ± 0.04 , 2.0 ± 0.16 , 722 ± 0.5 rayleighs (R). The absolute intensities are estimated to be accurate to $\pm 15\%$. We thus have an observed Ly- α /Ly- β intensity ratio of about 360 ± 54 . Calculated values of the scattering rates by the two lines of the multiplet give a ratio of about 590. We consider this good agreement in view of the preliminary nature of the analysis. Most of the uncertainty lies in the calculated relative scattering rates. We will have an opportunity to provide much improved estimates at the time of direct observation of the solar spectrum by the Voyager spectrometer.

The apparent underlying continuum shown in the spectrum of Fig. 1 is due to a combination of cosmic ray events and internal scattering of the optical spectrum entering the spectrometer aperture⁵.

There are some uncertainties in the calculation of the scattering rate factors or g values for the hydrogen lines. The most important of these are our knowledge of the relative solar line intensities over the averaged disk of the sun and the Ly- β solar line profile. In the near future it will be possible to improve on these values considerably by using the Voyager spectrometers to obtain simultaneous direct measures of the solar Lyman lines in the same time period as scattering observations. The Voyager spectrometer is capable of direct observations of the Sun at ranges greater than 3 AU. This observation will provide a reference g -value ratio appropriate to observations with the Voyager spectrometers.

Our ability to observe the hydrogen Ly- β emission line at 1,025 Å has important implications both for studies of the local interstellar medium and for the primary function of the Voyager experiment at the planetary encounters. It demonstrates the capability of obtaining measures of emissions as weak as 1 R.

Our ability to measure the pair of resonance lines will be useful in studies of the interplanetary and interstellar medium. Recent measurements from the Pioneer 10 spacecraft at 10 AU show a uniform Ly- α emission background of about 30 R (D. Judge, personal communication) and show no indication of correlation with galactic plane crossing. It has been suggested that practically all of this diffuse emission could be due to multiple scattering of solar Ly- α by interplanetary hydrogen (G. Thomas, personal communication).

If there is a multiply scattered component, the ratio of the intensities of the two H lines will differ from that of the singly scattered component due to the large transition probability ratio, $A_{\text{Ly-}\alpha}/A_{\text{Ly-}\beta} = 5.26$. The possible observation of multiply scattered solar Ly- α radiation is a year or more in the future for the Voyager spacecraft, because single scattering clearly dominates near the Sun. One method of separating the galactic and solar scattered components of Ly- α is to use the Ly- α /Ly- β ratio as a measure of the inclusion of the other sources.

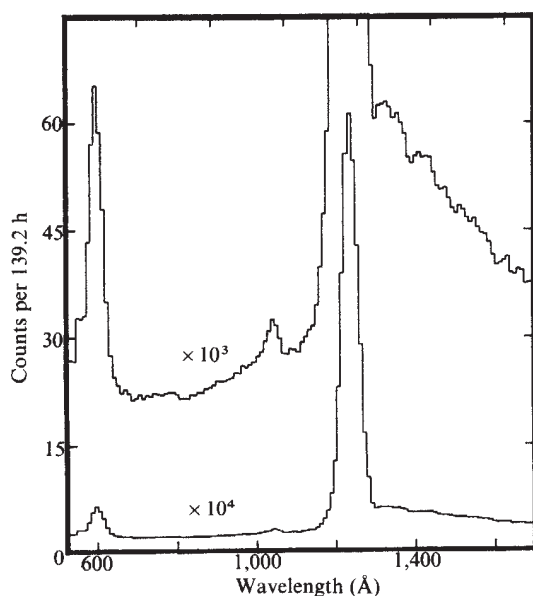


Fig. 1 Voyager 2 spectrum of the interstellar medium in the 500–1,700 Å region. The values, corrected only for detector threshold response, are the summation of 696 12-min spectra. The time intervals are not consecutive because of communication gaps and the occurrence of solar flare events. The mean observational direction was α 324°, δ -23°. The 1,216 Å feature is anomalously wide because of reflection near the edge of a filter in the detector structure.

The changing geometry of the observation as the spacecraft moves away from the Sun may permit separation of these sources. A galactic source will tend to be spatially patchy⁶ and is expected to be associated with the observed variation in scattered stellar continuum radiation⁷.

We cannot determine whether the observed emissions are entirely due to resonance scattered solar radiation from the available data. However, based on our experience with the Voyager spectrometers to date, we believe it will be possible to put strong limits on, or actually measure, an extrasolar component. We recently presented⁷ and discussed the first well defined measurements of the diffuse EUV radiation field in interplanetary space. The maximum observed emission rate at 975 Å was $\sim 0.06 \text{ R}/\text{Å}$ or $5 \times 10^3 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1} \text{ Å}^{-1}$ ($112^\circ \alpha$, $-13^\circ \delta$) and we obtain an interpolated value of $\sim 6 \times 10^3 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1} \text{ Å}^{-1}$ at 1,216 Å. This emission is presumably accompanied by some multiply scattered Lyman series radiation. Draine and Salpeter⁸ estimate a galactic Ly- α emission of less than 25 R in general with a maximum of about 100 R in the direction of some supernova remnants. The ability to separate a multiply scattered galactic source from the total Ly- α radiation will be enhanced by measures of Ly- α /Ly- β ratio in observed regions of high diffuse EUV radiation.

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On companions and comets

HARRISON¹ has recently hypothesised that the Sun possesses a companion star, in order to explain an anomaly in the distribution on the sky of pulsars which lose speed very slowly. This is a controversial suggestion mainly because proper motion sky surveys down to at least 14th mag (ref. 2) should have detected such an object. However, one can imagine various objects which might fulfill the constraint of not being visible. Harrison suggested that crystallised white dwarfs, red dwarfs and black dwarfs could be in a bound orbit about the Sun, and neutron stars and black holes would more likely be in an unbound orbit, due to the supposedly explosive natures of their births. Whatever the luminosities of these objects³ they must produce an acceleration of the barycentre of the solar system to remove the pulsar anomaly. We discuss here the effect of this acceleration on the orbits of the 'new' comets, and how it rules out the possibility of a solar companion in a bound orbit.

According to the Oort theory of comets⁴ a cloud of cometary material exists around the Solar System at a distance $\leq 10^4$ AU.

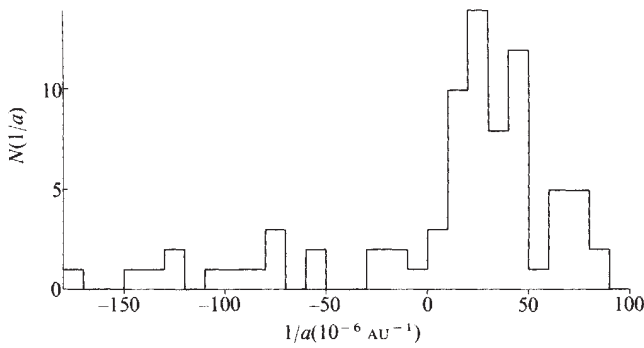


Fig. 1 The distribution of values of $1/a$ referred to the bary-centre of the Solar System for new comets.

This is a considerable part of the distance to the nearest star, but the cloud moves with the velocity of the Solar System and is therefore identified with the Sun. A new comet occurs when a part of this cloud is perturbed by a passing star which changes the perihelion distance of its orbit, bringing it into the range where it may become visible (≈ 6 AU). After experiencing the perturbation, the comet makes the long journey inwards to the Sun, taking a time approximately equal to the free-fall time $\sim 10^5$ yr to complete. Most of this time is spent in the outer part of the orbit at large distance and low velocity. We consider here the effect of a Sun's companion on the orbits of the comets in two cases: for a companion in a bound orbit, and for a companion in an unbound orbit.

In a bound orbit the motion of the comet is an excellent approximation of the motion in the restricted problem of three bodies⁵. We consider a companion of mass $M = M_\odot$ in the circular orbit at a distance d from the Sun, and describe the motion of a comet in the frame rotating with the Sun and companion. The position of the Sun is denoted by the vector $+\mathbf{d}/2$, the companion by $-\mathbf{d}/2$, and that of the comet by $\mathbf{r}$. Then the velocity, $\mathbf{V}$, in the non-rotating system is given in terms of the velocity $\mathbf{v}$ in the rotating system and the angular velocity vector $\boldsymbol{\omega}$ by

$$\mathbf{V} = \mathbf{v} + \boldsymbol{\omega} \wedge \mathbf{r} \quad (1)$$

A comet in the Oort cloud which has just started its journey to the Sun has an energy of the same order of magnitude as its gravitational binding energy,

$$E = \left[\frac{1}{2} V^2 + U(\mathbf{r}) \right]_{\mathbf{r}=\mathbf{R}} = O\left(\frac{GM}{R}\right) \quad (2)$$

where $U(\mathbf{r})$ is the gravitational potential, and R is a radius typical of the Oort cloud. The Jacobi integral of motion for this system is

$$J = \frac{1}{2} v^2 - \frac{1}{2} (\boldsymbol{\omega} \wedge \mathbf{r})^2 + U(\mathbf{r}) \quad (3)$$

and its value according to equations (1) and (2) is

$$J = -[\mathbf{V} \cdot \boldsymbol{\omega} \wedge \mathbf{r}]_{\mathbf{r}=\mathbf{R}} + O\left(\frac{GM}{R}\right) \quad (4)$$

At perihelion, we can measure the reciprocal of the semi-major axis of the comet's orbit by adding the apparent kinetic energy of the comet to the apparent potential energy

$$E_{\text{obs}} = \frac{1}{2} v^2 + U\left(\frac{d}{2}\right) + \frac{GM}{d} \quad (5)$$

This is related to the reciprocal major axis by

$$\frac{GM}{2a} = -E_{\text{obs}} \quad (6)$$

From equations (3) and (5)

$$E_{\text{obs}} = J + \left[\frac{1}{2} (\boldsymbol{\omega} \wedge \mathbf{r})^2 \right]_{\text{perihelion}} + \frac{GM}{d} \quad (7)$$

So that from equations (4) and (6) we obtain

$$\frac{1}{a} = \frac{2}{GM} \left\{ [\mathbf{V} \cdot \boldsymbol{\omega} \wedge \mathbf{R}]_{\text{Oort-cloud}} + O\left(\frac{GM}{R}\right) - \left[\frac{1}{2} (\boldsymbol{\omega} \wedge \mathbf{r})^2 \right]_{\text{perihelion}} - \frac{GM}{d} \right\} \quad (8)$$

Here the first term is normally the dominant one, so that if we assume that a comet may originate in any part of the Oort cloud we would expect to observe a distribution of values of $1/a$ having an average value:

$$\left\langle \frac{1}{a} \right\rangle \approx -\frac{2}{d} \approx -2 \cdot 10^{-3} \text{ AU}^{-1}$$

and a spread

$$\left\langle \left(\Delta \frac{1}{a} \right)^2 \right\rangle^{\frac{1}{2}} \approx \omega \left(\frac{R}{GM} \right)^{\frac{1}{2}} \approx 10^{-2} \text{ AU}^{-1}$$

Figure 1 displays the observed distribution of $1/a$ for 77 new comets⁶. The average is approximately $1.1 \times 10^{-5} \text{ AU}^{-1}$ and the spread $5 \times 10^{-5} \text{ AU}^{-1}$. From this we conclude that the Sun does not possess a bound companion of this kind

For a companion in unbound orbit the problem is slightly different, because the time during which the comets feel a perturbation depends on the velocity of the companion. An interesting possibility is that an object of the proposed galactic halo is passing close to the Sun, in which case one could guess from the virial theorem that the velocity would be around 150 km s^{-1} . Such an object would pass all the way across the Oort cloud in only 200 yr. Its effect on a comet in the distant part of its orbit would not be seen until this comet reaches the Sun in 10^4 – 10^5 yr time. Here we calculate the effect on comets which are already within 100 AU of the Sun. A comet of 100 AU takes approximately 70 yr to reach perihelion; this corresponds to the time during which the companion would be within about $3 \times 10^3 \text{ AU}$ of the comet. For the perturbation equation we have⁷:

$$\frac{d}{dt} \left(\frac{1}{a} \right) = -\frac{2}{h} \left(S \frac{p}{r} + R e \sin \theta \right) \quad (9)$$

Here a is the semi-major axis of the comet's orbit, h is the specific angular momentum, S and R are the gradients of the perturbing function in the azimuthal and radial directions respectively, e is the eccentricity, r and θ the comet's position in plane polar coordinates with the Sun as origin and θ measured from the direction of perihelion, and p is the semilatus rectum;

$$p = a(1 - e^2) \quad (10)$$

First, however, we note three points relevant to the Sun-unbound companion-comet system: (1) the orbit of the companion is approximately a straight line along which the velocity is constant, as the kinetic energy is much greater than the modulus of the potential energy. In making this approximation we exclude the possibility that the companion has already passed much closer than 10^3 AU to the Sun, and do not include companions of low velocity. For these objects, which

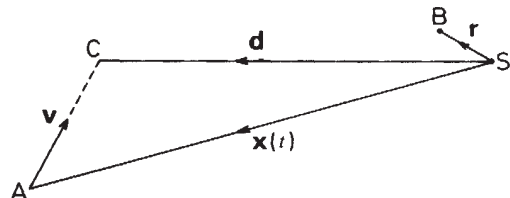


Fig. 2 AC is part of the orbit of the companion. At $t=0$ the companion is at C, the Sun is at S, and $CS \sim 10^3 \text{ AU}$. At time $t(t < 0)$ the companion is at A, $\mathbf{x}(t) = \mathbf{d} + \mathbf{v}t$. The comet is at B, with position vector $\mathbf{r}$.

are just unbound, a much larger perturbation of the cometary orbits is expected, as they are closer to the Sun for a longer time. (2) When the comets in which we are interested experience the perturbation they are much closer to the Sun than is the companion. We therefore use the Sun-companion distance instead of the companion-comet distance to calculate the perturbation. (3) Comparing the effect of the perturbations S and R on $1/a$, the S term includes a factor

$$\frac{p}{r} = \frac{a(1-e^2)}{r} = \frac{2q}{r} \quad (11)$$

where q is the perihelion distance. On the other hand, the R term includes a factor $e \sin \theta$ which is zero at perihelion. Hence S is the only important perturbation when $q \sim r$, and R is the only important perturbation when $r \gg q$. Even when within 100 AU of the Sun, a comet spends only a small fraction of its time with $r \sim q$, so that we can neglect the effect of S . We further replace $e \sin \theta$ by $1/5$, as θ varies slowly for $r \gg q$ and $e \sin \theta \approx 1/5$ for $r = 100$ AU and $q = 1$ AU in a parabolic orbit.

The result of these approximations is

$$\left| \frac{d}{dt} \left(\frac{1}{a} \right) \right| = \frac{2}{h} R(t) \quad (12)$$

where

$$R(t) = \frac{r}{x^3} (1 + [\hat{\mathbf{x}} \cdot \hat{\mathbf{r}}]^2) \quad (\text{see Fig. 2})$$

From equation (12) we estimate the mean square variation in $1/a$ as

$$\left\langle \left(\frac{1}{a} \right)^2 \right\rangle^{1/2} = \frac{2}{5} \frac{1}{h} \frac{GM}{d^2 v} \quad (13)$$

where we have ignored the time dependence of r .

The comets in Fig. 1 have r.m.s. variation

$$\left\langle \left(\frac{1}{a} \right)^2 \right\rangle_{\text{obs}}^{1/2} = 50 \quad (10^{-6} \text{ AU}^{-1}) \quad (14)$$

Equating (13) and (14) gives the velocity of the companion

$$v = 20 \text{ km s}^{-1} \quad (15)$$

This may be regarded as a lower limit for v , as the $\langle [\Delta(1/a)]^2 \rangle_{\text{obs}}^{1/2}$ can be adequately explained in the conventional theory⁴.

In conclusion, the orbits of the new comets are so close to being parabolic that the existence of a companion in bound orbit about the Sun can be excluded. Furthermore, this sets a lower limit on the velocity of a companion in hyperbolic orbit about the Sun. This lower limit is, however, insufficient to rule out the possibility that the Sun is undergoing an encounter with an object from the galactic halo.

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Width of a planetary ring system and the C-ring of Saturn

PLANETARY ring systems such as those of Saturn and Uranus seem to have an inner limit analogous to the Roche outer limit. The analysis of the C-ring of Saturn described here gives an

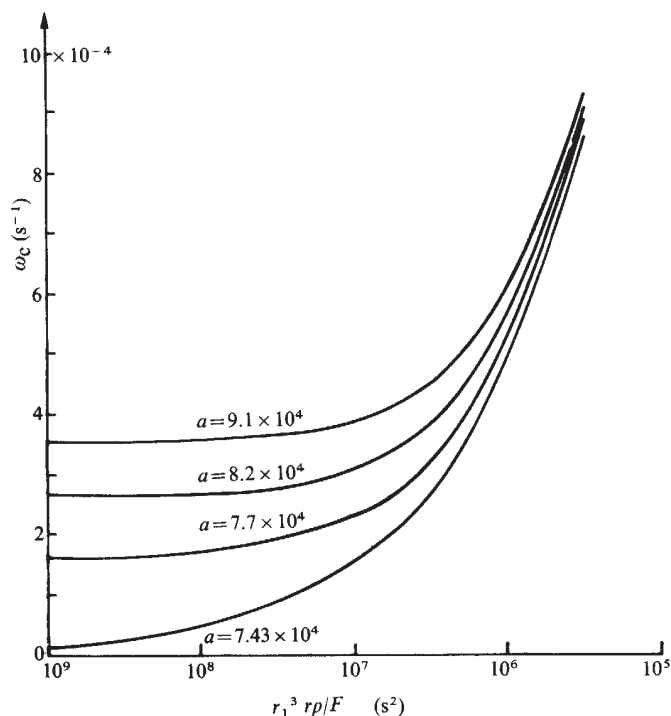


Fig. 1 Critical angular velocity ω_c as function of radii of the interacting particles for various orbital radii a (in km): for $\omega > \omega_c$ a small particle (r_1) will escape from the surface of a large one (r). F is a non-gravitational attractive force, ρ is density.

insight into its structure and dynamics. The quasi-steady distribution of particle sizes in a ring requires that besides collisional comminution and sputtering by protons, electrons and meteorites¹ there are processes which permit the particles to grow. On Saturn, one of these processes is condensation² on to the inner rings of water molecules sputtered from the outer edge³ of ring A. Another is the adhesion of smaller particles to the larger ones after low velocity collisions produced by gravitational attraction and by the differences in Keplerian velocities⁴. This process can include ejecta formed during cratering. The question arises under what condition these small particles will stay long enough on the surface of the larger ones to develop a permanent bond.

First, we assume that the only force acting between the particles is gravitational attraction. Let a be the radius of the orbit of a particle of radius r and density ρ around a planet of mass M and let a small particle rest on the outwards trailing sector of the orbiting particle at a distance m beyond its orbit. Then the small particle is subjected to a net outward acceleration:

$$D_1 = GM[(a+m)a^{-3} - (a+m)^{-2}]$$

The particle is also subjected to a gravitational attraction by the larger particle which has a component $D_2 = 4\pi G \rho m / 3$ opposing D_1 . It follows that the small particle will escape if $D_2 < D_1$, that is, if $4\pi \rho a^3 / 3M < 3 - 3p + 4p^2 - 5p^3$ and so on, where $p = m/a \ll 1$. Typical paths of such particles have been numerically calculated by D. R. Davis (personal communication). For $m = r$ the condition for escape from this (and from the leading inward) sector of the large particle is $a < a_c = (9M/4\pi\rho)^{1/3}$ which for Saturn and $\rho = 1$ gives 7.43×10^4 km. Small particles will stay on the surfaces of the remaining two sectors for all values of a . In a ring which is dense enough, particles undergo collisions, however, and rotate so that the above condition for escape applies essentially to the whole surface of the orbiting particles. The tendency to escape depends on m and this could be important for synchronous particles by increasing their non-sphericity. The orbital radius a_c falls within the range of uncertainty⁵ of the inner edge of ring

$C\ 7.68 \pm 0.3 \times 10^4$ km (suggested in ref. 4) which indicates that no substantial rings can exist without the ability of particles to grow. At radii smaller than the inner edge of ring C, that is in the D ring, the only possible mechanism of growth is condensation of water molecules² which can be instantaneously bonded. The width of a planetary ring is thus given by the requirement that

$$(9M/4\pi\rho) < a^3 < (M/\pi c\rho) \sim (11M/\pi\rho) < (18M/\pi\rho)$$

where c , as given by Roche, is 0.090093. (For two equal particles $a^3 = 18M/\pi\rho$ which lies just outside ring A.)

Forces other than the gravitational one and the rotation of the particles around their axes have an important additional effect. As one expects the rings to be surrounded by a neutral low velocity plasma², it is reasonable to assume that, on average, the ice particles are neutral so that no significant electrostatic forces act between them. On the other hand, van der Waals attraction F is always present. If the orbiting particle rotates with an angular velocity ω then there is a critical ω_c given by

$$\omega_c^2 = (4/3)\pi G\rho - 3MG/a^3 + (3/4\pi\rho)(F/r_1^3r)$$

such that for $\omega > \omega_c$ a small particle with radius r_1 escapes from the surface of the larger one. Figure 1 shows the dependence of ω_c on $r_1^3\rho/F$ for various radii a within the width of the C ring. It follows that, for a given ω_c and F , particles of radius r can grow to a maximum value of $r_1^3r_{\max}$ which increases with increasing a or, in other words, for a given a , a particle with radius r_1 can attach itself to a particle with radius r only if the latter does not rotate too rapidly and if r is smaller than $r_{\max}$ which depend on a . At the inner edge of ring C, that is at a_c , a rotation velocity of 10^{-3} s^{-1} would prohibit the growth of all but the smallest particles.

Greenberg *et al.*⁴ concluded that the number of particles of size r is given by $\alpha r^{-\beta}$ where α is a constant and β is ~ 3 . Thus, if one assumes that the C ring is thin enough for mutual shadowing to be ignored, the contribution to opacity by particles of size r is roughly proportional to r^{-1} and the total opacity at a given a is proportional to $\log(r_{\max}/r_{\min})$. For a given r_1 and F , $\log r_{\max}$ is approximately proportional to a if ω_c is $4\text{--}5 \times 10^{-4}$ which may account for the observed linear increase of opacity with a . This explanation is, however, not unique and other assumptions about shadowing and about $\omega_c(a)$ could lead to similar results. The choice of an appropriate value of F is difficult because the experiments and the theory aim at almost ideal surfaces, while the icy particles in the rings are undoubtedly irregular and rough even if they are covered with amorphous ice². Such particles will touch in not more than three or four places in each of which a few atoms are close enough to develop a van der Waals interaction. Experiments on very good surfaces^{7,8} give forces of the order $10^6\text{--}10^{10}$ dyne cm^{-2} . A contact area of the order of $1,000 \text{ \AA}^2$ gives $F = 10^{-5}\text{--}10^{-7}$ dyne with an error of two orders of magnitude. With $F = 10^{-6}$ dyne and $\omega = 3 \times 10^{-4} \text{ s}^{-1}$ the maximum value of r_1^3r at $a = a_c$ is 2 cm (ref. 4) which agrees with the conclusion that particles smaller than about 1 cm would be removed from the rings by the Poynting–Robertson effect⁵.

The rotational velocity of the ring particles can be estimated by assuming that the rotational energy E_R of all pairs of particles which can collide (that is, $r_1 + r_2 = \Delta a$) is comparable with their relative translational kinetic energy E_T for velocity difference $(\Delta v)^2 = (GM/4a^3)(\Delta a)^2$. With $E_R = (4\pi\rho/15)\omega^2\alpha(r_1^3 + r_2^3)$ and $E_T = (\pi\rho GM/6a^3)\alpha(r_1 + r_2)^2$ after averaging over all r one obtains

$$\bar{\omega}^2 = (3/8)(GM/a^3)(1 + 2 \log 2) \quad \text{or} \quad \bar{\omega} = 2 \text{ to } 3 \times 10^{-4} \text{ s}^{-1}$$

which is comparable with ω_c . The maximum possible radius of a particle rotating with this angular velocity, which corresponds to about one turn per orbit, is given by $(r\omega)^2\rho = 8\sigma(1-\nu)(3-2\nu)^{-1}$ where σ is the maximum strength of the solid and ν is the Poisson's ratio. Putting $\sigma = 3 \times 10^6$ to 3×10^7 dyne cm^2 and $\nu = 0.3$ one obtains $r = 70\text{--}90$ km which is close

to values calculated from the tidal effect⁴. For $\omega > 5.25 \times 10^{-4} \text{ s}^{-1}$ (see ref. 9) and $F = 0$ no accretion is possible¹⁰ at any a .

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Brightness oscillations of daytime sky

WE report here the discovery of sky brightness oscillations, observed during May 1976 and November 1977, which can be associated with atmospheric waves, perhaps on the aerosol boundary layer; this discovery also has a bearing on experiments now investigating solar diameter oscillations.

One purpose of our measurements has been to detect oscillations associated with the brightness changes of the Sun, related, for example, to the well-known 5-min period of photospheric spectral line variations. Two-colour brightness and polarisation data were obtained while our polarimeter¹ was attached to the 0.75 m reflector telescope at the South African Astronomical Observatory (SAAO), Sutherland, in May 1976. Inspection of the brightness data from the zenith sky revealed fluctuations above photon shot noise which were superimposed on the general diurnal changes caused by the movement of the Sun across the sky. As the gradients of the diurnal changes are steep and tend to mask the more rapid fluctuations, their effect was removed to a large extent by fitting a second order polynomial to the data by the method of least squares; such a curve gives a good fit to the data for the middle part of the day. The residuals showed an oscillation with a frequency which was real to the data, clearly too high to have been generated by the curve fitting procedure. A subsequent Fourier analysis gave the period as 27 min with an amplitude stronger in the red channel than in the blue. Although a visual inspection of the polarisation data sometimes gave a hint of small oscillations in antiphase, they were not apparent after the same curve fitting and Fourier analysis procedures.

An opportunity to confirm the daytime sky brightness oscillation phenomena occurred in November 1977 when our photometer² was attached to the Morgan reflector (0.6 m) at the Lowell Observatory, Flagstaff, Arizona. This instrument includes a polarising beam splitter to allow simultaneous two-colour photometry; the data from each channel correspond to the brightness of one of the Stokes linear polarisation parameters, full polarimetry not being available. However, the polarising properties of the photometer were put to good effect by setting the orientation of the mounting plate of the telescope so that the diurnal brightness changes were compensated to a large extent by the rotation of the direction of vibration of the polarised light from the sky during the observing run. This procedure reduces the apparent brightness gradients and makes any oscillations more readily visible.

The observations at Flagstaff immediately revealed oscillations of the brightness of the zenith sky (see Fig. 1), their magnitude being slightly larger than 1% of the recorded signal, and hence, ruling out a direct solar origin. This conclusion was confirmed by simultaneous records obtained with a second

photometer belonging to the University of Washington attached to the 0.75 m telescope at Lowell Observatory's outstation at the Anderson Mesa some 25 km from Flagstaff; there was no coherence in the signals from the two stations. A similar two-station experiment was attempted between Flagstaff and Mt Lemmon, Arizona, where the data at the latter site were obtained by our polarimeter but the presence of cirrus cloud at either one of the sites prevented simultaneous comparison.

The form of the oscillations is very complex; any observed waveform may persist typically for three to five cycles before being replaced by another of different form and frequency. The oscillations have a broad spectrum of periods from minutes to the order of an hour. The general behaviour of the signal variations is similar to the phenomena observed in microbarograph records³ and we suggest that these newly discovered brightness oscillations reflect the presence of atmospheric gravity waves causing the air mass in the zenith column to fluctuate.

Both atmospheric absorption and refraction are known to display similar oscillatory phenomena. For example, fluctuations of air mass along the direction to the Sun have been reported by Fossat *et al.*⁴ who measured variations of the strength of telluric lines in the solar spectrum and KenKnight *et al.*⁵, who have recorded fluctuations in differential refraction which contribute noise to their astrometric programme. It may also be noted that the latter workers conclude that the power in the differential refraction fluctuations across half a degree is of the same order as that observed by Hill *et al.*⁶ in their solar diameter measurements, hence, giving an alternative explanation to Hill's suggestion that the Sun's disk is itself oscillating. By translating the observed air mass variations into refraction fluctuations, Fossat *et al.*⁴ have also challenged the validity of Hill's conclusions; if an equivalent translation is made for the sky brightness oscillations reported here, a similar challenge to Hill can be made (see ref. 7).

From the accumulated data, the amplitudes of the oscillations recorded at Mt Lemmon (2,776 m) and Flagstaff (2,204 m) are larger than at Sutherland (1,768 m), suggesting that they originate fairly high in the atmosphere. At a lower altitude site, the greater part of the observed brightness is generated by scattering from the lower and more dense parts of the atmosphere giving a strong background level against which the fluctuations are to be detected—but the effects of weather patterns, season and latitude may also be important.

The two-colour brightness oscillations recorded at Flagstaff were strongly coherent, with the amplitudes being just less than twice as large for the red as for the blue; this is explained by the fluctuations occurring at a particular height in the atmosphere, without the whole zenith column being affected. For the most simple models, for a given oscillation of the air mass of the zenith column, one expects an identical fractional change in brightness at all wavelengths. By introducing absorption (selective) to the model describing the downwards scattered light, any amplitude observed in the blue is suppressed relative to the red by the order of 1.1, that is, the sense is right but the numerical term is too small. If a simple atmosphere is considered comprising air molecules with a $1/\lambda^4$ scattering efficiency, and aerosols with a scattering efficiency which is independent of wavelength, then the ratio of amplitudes for the two colours (red/blue) would be approximately 3.34 for a change in the aerosol content alone, assuming that the aerosols typically contribute ~5% of the observed brightness (for example, ref. 8). However, if this is diluted by a coherent change in the number of air molecules, but not to the same degree, the value of the amplitude ratio can be readily engineered to below 2. For example, an air molecule change of 0.5% accompanied by an aerosol change of 20% would provide a change of red brightness of 1.5% and a red/blue amplitude ratio of 1.8, which matches well with the observed phenomena. Because the scattering by aerosols reduces the high polarisation produced by the scattering from air mole-

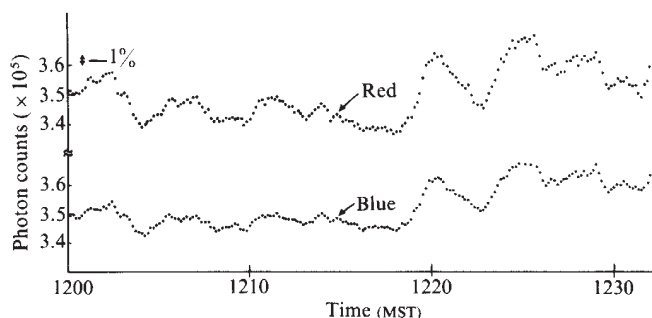


Fig. 1 A specimen set of observations depicting zenith sky brightness oscillations at Flagstaff on 8 November 1977, with the integrated photon count (10 s per data point) plotted against mountain standard time (MST). To demonstrate the difference in amplitude of the fluctuations between the red and blue channels, the counts for the blue channel which provided a higher count rate have been normalised. A diaphragm in the telescope focal plane limited the field of view to 15 arc s; the central wavelengths of the passbands were set by tilting narrow band interference filters to 6,540 Å (2 Å passband, FWHM) for the red channel and to 4,840 Å (2.5 Å passband) for the blue.

cules, with such a change in the air column one would expect oscillations to be apparent in the recorded polarisation values. The detection of such polarisation oscillations has already been referred to and although no definite correlation has been established, the fact that the possible weak polarisation fluctuations may be in antiphase with the brightness oscillations is perhaps significant. A simple calculation using the percentage changes above and assuming that the flux from aerosols is unpolarised suggests that, accompanying a typical brightness increase (aerosol content increase), the polarisation should decrease by a few tenths of 1%, this being of the same order as the photon error on the polarimetric measurements.

This explanation of the observed colour dependence of the brightness oscillations may suggest that they result from corrugations on a boundary layer, a possible source of gravity waves⁹, which is trapping the main aerosol content beneath it and as the waves pass by, the aerosol content along the zenith column has a large fluctuation in relation to the air mass.

Future experiments to investigate this proposal should involve higher accuracy polarimetry and observations at more wavelength values. To obtain information on the size of the waves and their height, it would be useful to investigate the coherence of the oscillations over the sky from both a single station and pairs of stations. Coherence with a microbarograph might also be investigated.

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A consistent age for the Universe

THE age of the Universe is one of the most interesting cosmological parameters. The fact that the age is finite has intriguing philosophical as well as physical implications. An accurate age when compared to the present expansion rate parameter, the Hubble constant H_0 , can in the standard big bang model even determine the large scale structure of the Universe, that is decide whether the Universe is open or closed. This note combines the various constraints of the traditional dating methods in the context of the standard cosmological model to see which age can satisfy them all. This simultaneous solution is more restrictive than the previous ranges on the age of the Universe, and agrees with the description of the Universe given by Friedmann.

Three independent methods¹ have traditionally been used to determine the age of the Universe, T_u . These are: (1) using the dynamics of the Friedmann Universe², in particular, estimates of H_0 and the limits of the deceleration parameter q_0 ; given that published values for H_0 range from 40 to 100 km s⁻¹ Mpc⁻¹, and limits on q_0 range up to ~ 1 , the most restrictive age range on which everyone will agree, from this method, is 5,000 $\leq T_u \leq 25,000$ Myr. (2) Using the ages of the oldest stars, in particular, the ages of globular clusters. Assuming that the time from the big bang to star formation is $\ll 1,000$ Myr, globular cluster ages³ then give 8,000 $\leq T_u \leq 18,000$ Myr where the main uncertainty is in the helium abundance. (3) Using nucleochronology, that is direct dating of the nucleosynthesis of radioactive nuclei from their observed or implied abundances. Independently of a model, one obtains the mean age of the elements⁴, which is a lower limit to the age of the Universe. This gives⁵ $T_u \geq 6,000$ Myr for the absolute lower limit, with mean ages ranging from 6,000 to 10,000 Myr. By coupling the mean age to a galactic evolution model a total age is obtained for the heavy elements which should be approximately the age of the Universe. Most models of galactic evolution which do give the correct mean age (which is known independently of a particular model) have a total age which implies that $T_u \leq 20,000$ Myr (ref. 5).

It is surprising that three such diverse methods give roughly the same range. Unfortunately, the uncertainty in the age given by each of these methods is large. An interesting point is that by combining two or more of the methods and inserting constraints from related observations, a much more restrictive determination of the concordant age is possible. This method was used in part by Gott *et al.*⁶ who determined that the age must lie in the range 13,000 $\leq T_u \leq 20,000$ Myr. The lower limit on this range was determined by the need for concordance between the deuterium-limited density $\rho = 5 \times 10^{-31}$ g cm⁻³, the lower limit on the density parameter $\Omega = \rho/\rho_c \approx 0.06$ (where $\rho_c = 3H_0^2/8\pi G$ is the critical density) implied from dynamics of galaxy groups and clusters and because the age from the dynamical method is totally specified for the standard big bang model (with cosmological constant, $\Lambda = 0$) if ρ and Ω are given, since the simultaneous knowledge of each determines H_0 and q_0 . The upper limit in ref. 6 comes from either the age of globular clusters or nucleochronology plus galactic evolution.

Another method of obtaining a concordant age is by using the fact that the ages of globular clusters depend on the primordial helium abundance. As the origin of this helium was presumably big bang nucleosynthesis, and as the amount of helium produced in the big bang is sensitive to the baryon density, ρ , one can use this density to constrain q_0 which, coupled with H_0 , determines the age from dynamics. This constrained age must also be concordant with the globular

cluster age. This method gives concordant ages near 15,000 Myr (refs 7, 8).

We assume that on large scales the Universe is homogeneous, isotropic, and at present, matter-dominated with negligible pressure. All these assumptions are supported by observations and hence do not seem to introduce any serious ambiguity. If one then considers the simplest of these models, namely those with zero cosmological constant Λ (an assumption whose consistency must eventually be checked with observations) two parameters are sufficient to completely specify a particular model.

First, the Hubble constant

$$H_0 = \frac{\dot{R}_0}{R_0} \quad (1)$$

where $R = R(t)$ is the scale factor of the Universe and R_0 is its present value.

Second, the density parameter, $\Omega = \rho/\rho_c$ which is related to the deceleration parameter

$$q_0 = -\frac{R_0 \ddot{R}_0}{(\dot{R}_0)^2} \quad (2)$$

by

$$\Omega = 2q_0 \quad (3)$$

For the $\Lambda = 0$ models, the value of Ω determines whether the Universe is open ($\Omega \leq 1$) or closed ($\Omega > 1$). With the above assumptions the age of the Universe can be given in terms of the observable quantities Ω and H_0 by

$$T_u = \frac{F(\Omega)}{H_0} \quad (4)$$

where $F(\Omega)$ is given in refs 2 and 6. The age of the Universe is, therefore, constrained within the $\Lambda = 0$ Friedmann models by the measurements of Ω and H_0 . Consequently, accurate determination of any two of these parameters will set limits on the third which will have to be consistent with observations.

Once the above model for the Universe has been assumed, one must conclude that in an earlier era its temperature T has been high enough for nucleosynthesis to occur. Such a route for nucleosynthesis has been explored in detail by Wagoner *et al.* (ref. 9, and for review, ref. 8). The main conclusion obtained from big bang nucleosynthesis is that the predicted ⁴He abundance agrees well with observations and the yields of both ⁴He and D depend on the entropy per baryon, $h_0 = \rho/T^3$, in the Universe and hence on Ω . Using equation (4) and the results of big bang nucleosynthesis, each model of the Universe can, therefore, be alternatively specified by T_u and Y , the primordially produced ⁴He abundance. The different models spanning the T_u , Y space are shown in Fig. 1 (see also refs 7 and 8). The different curves correspond to models of the same H_0 , which ranges between 40 $\leq H_0 \leq 100$ km s⁻¹ Mpc⁻¹ (refs 19, 20) thus, excluding all models with H_0 outside this range. Our conclusions do not, however, explicitly use H_0 as input.

The class of allowed models can be further restricted by estimating the contribution of matter associated with galaxies, to the density parameter (whose values run along the $H_0 = \text{constant}$, lines in Fig. 1). The best values of Ω determined from the dynamics of galaxies, give values in the range 0.06 $\leq \Omega \leq 0.3$ (refs 6, 10, 11) independent of H_0 . These values give a lower limit of Ω , as they do not take into account any contribution from intergalactic matter, whose existence has not been conclusively shown⁶. These values of Ω , and the observed deuterium abundance, X_D , can now be used as a consistency check with observations, as X_D depends strongly on the value of baryon density, ρ , and hence on Ω . The most recent values of

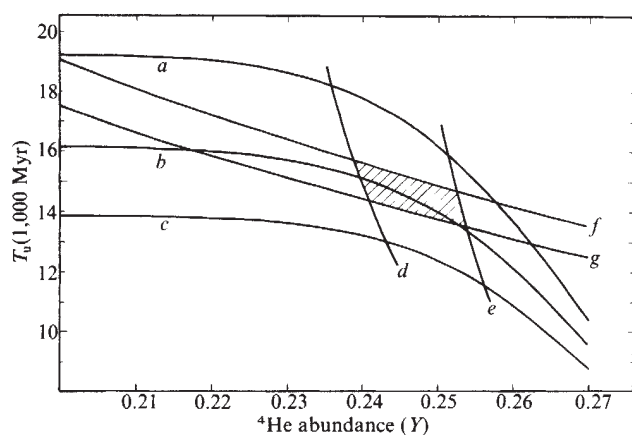


Fig. 1 Constraints on the age of the Universe, T_u , and the primordial produced ${}^4\text{He}$ abundance. Curves of constant H_0 : a, $H_0 = 50$; b, $H_0 = 60$; c, $H_0 = 70$ and of constant density parameter Ω : d, $\Omega = 0.06$; e, $\Omega = 0.3$, according to the standard big bang nucleosynthesis model. Ages of the globular clusters are given for different primordial heavy element abundances Z : f, $Z = 10^{-4}$; g, $Z = 10^{-3}$.

the deuterium abundance are those of Dupree *et al.*¹² obtained with the Copernicus satellite. These range from $D/H = 3.9 (+5.7, -1.7) \times 10^{-5}$ and $0.24 (+0.12, -0.07) \times 10^{-5}$ in the directions of α Aur and α Cen A, respectively. If other sources have not contributed to the deuterium abundance¹³, and as deuterium has certainly been destroyed in stars, the primordial deuterium abundance should be greater than the largest of the observed lower bounds, that is $D/H \geq 2.2 \times 10^{-5}$, or $X_D \geq 3.4 \times 10^{-5}$, assuming $X_H = 0.768$. The corresponding limit on Ω is then given by⁸

$$\Omega \leq 0.085(50/H_0)^2 \quad (5)$$

As Ω ranges between ~ 0.06 and ~ 0.3 , the corresponding limit on H_0 is then $H_0 \leq 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This value of H_0 agrees with those observed, again showing the model to be compatible with observations. (However, it disagrees with the speculations of ref. 14 on a large value of H_0 .)

If it is assumed that the time from big bang to star formation is much less than 1,000 Myr, then the ages of the oldest stars would set limits on the age of the Universe. Estimates of the globular cluster ages can be made, and are based on detailed stellar evolution models. Such an analysis concluded that their ages are strongly dependent on the helium abundance Y , and more weakly on the heavy element mass fraction Z , of the gas out of which they have been formed^{3,15}. As globular clusters are very old, their helium abundance at birth must not be too different from that of helium synthesised in the big bang. Their ages (in units of 10,000 Myr) are then given in terms of Y and Z by³

$$\log_{10} t_c \approx 0.035 + 2.085(0.3 - Y) - 0.034(\log_{10} Z + 3) \quad (6)$$

with $10^{-3} \geq Z \geq 10^{-4}$. This result is essentially independent of the distance to globular clusters because the turnoff luminosity was obtained relative to that of the horizontal branch, and the relative luminosity provided the necessary model normalisation. The most metal-poor globular clusters appear to have $Z \approx 10^{-2} Z_\odot$ or 1.7×10^{-4} (ref. 17), while those with $Z \geq 10^{-3}$ all have young 'turnoff' ages¹⁸, and are, therefore, not the old objects we seek. The value of the cluster age, t_c , has been

plotted in the T_u , Y plane, as a function of the helium abundance Y , with $10^{-3} \geq Z \geq 10^{-4}$. This constraint further restricts the concordant models of the Universe. Demarque and McClure¹⁶ have calculated ages of globular clusters in a way that seems to be almost independent of Y . (However, this is done only by building into their ages an implicit determination of Y from fitting the ratio of the number of red giants to horizontal branch stars, which has several uncertainties built into it). The ages they find range between 15,000 and 18,000 Myr—ages within the range allowed by Iben.

As mentioned above, another completely independent way for determining the age of the Universe is by nucleochronology plus galactic evolution. These limits are not very stringent due to uncertainties in galactic evolution models. In addition, there are uncertainties in the model-independent mean age due to uncertainties in various nuclear parameters. In particular, more accurate measurements of the half life of ${}^{187}\text{Re}$ will significantly restrict the range of values deduced by nucleochronology and provide a very important consistency test of the various models. Even with the uncertainties in galactic evolution, the mean age of the elements is a firm lower limit on T_u .

By incorporating all the above discussed constraints on the T_u , Y diagram (Fig. 1), the allowed age of the Universe completely consistent with them is quite restricted. It ranges from 13.5 to 15.5×10^9 yr, determined by the limits $0.06 \leq \Omega \leq 0.3$, and $10^{-4} \leq Z_{g.c.} \leq 10^{-3}$. These limits are more restrictive than those obtained from the individual constraints and, as argued earlier, consistent with the independent measurements of H_0 , and those of q_0 . The limits on H_0 along with the deduced age, T_u , are consistent with an open model of the Universe⁴. If the age of the Universe were eventually found to lie outside this narrow range, then one would be forced to question the standard cosmological model and/or standard globular cluster models both of which are frequently taken as illustrations of the success of modern astrophysics.

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On the nucleation of the martensite transformation

THE standard description of the austenite–martensite transformation includes the statement that once the material reaches the martensite start temperature, or below, then the transformation occurs immediately¹. Hence, if a nucleus is present in the material, then growth occurs to an extent determined by the transformation temperature. We expect, therefore, that there should be no time delay in this transformation and that the amount of martensite should depend on the transformation temperature, for a given grain size and composition². If there is a time delay in the appearance of martensite, again at a temperature at or below the martensite start temperature (M_s), then it is important to show this by establishing that no growth of martensite occurs while a specimen is held at or below M_s . We show here that the nucleation event and not the growth behaviour of martensite can be affected by a common metallurgical treatment. The experiment is carried out at a constant grain size and composition with the result that we can unambiguously relate the change in nucleation rate to the present procedure.

The experiment was carried out as follows: samples of Fe–30% Ni were arc melted into rods, swaged to a final size of 0.7 mm and then annealed at 1,200 °C for 20 h. In this way we obtained a constant grain size and also homogenised the structure. Thin samples, treated as described, were cooled from 1,200 °C to a series of temperatures, held another 2 h at the respective temperatures and then quenched to room temperature. Samples treated in this way were subsequently cooled to temperatures at and below M_s , held for various lengths of time and then kept at room temperature for various observations. While samples were cooled to the respective transformation temperature—typically the bath was a precooled alcohol bath—the acoustic emission which accompanied the transformation was recorded. This procedure ensured that: (1) the only independent variables in the procedure were the temperature the sample was quenched from, the temperature the sample was transformed at and the time at this temperature. All other variables were held constant. (2) The recorded acoustic emission signal registered the time necessary for the transformation to start. This, as we shall show, was a yes or no situation because when an acoustic emission signal is recorded, then martensite can be observed by optical microscopy. Conversely, if an acoustic emission signal were not recorded, then no martensite platelets would be observed in those samples.

These experiments gave the following results. The acoustic emission which accompanies the austenite–martensite transformation is found to be a function of time at transformation

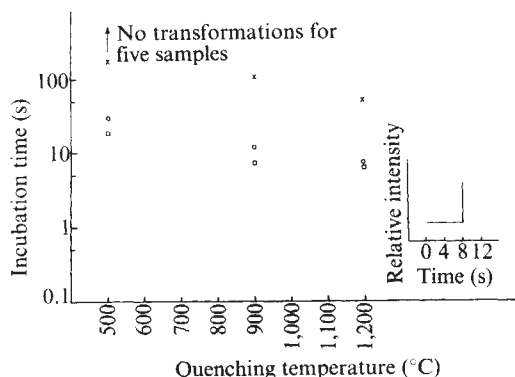


Fig. 1 Incubation time, as defined in inset for various samples which have been quenched from various high temperatures. The inset shows a typical acoustic emission pattern for Fe–30% Ni sample previously heated to 1,200 °C for 20 h then quenched to room temperature and transformed at –28 °C. Transformation temperatures: x, –25 °C; o, –28 °C; □, –30 °C.

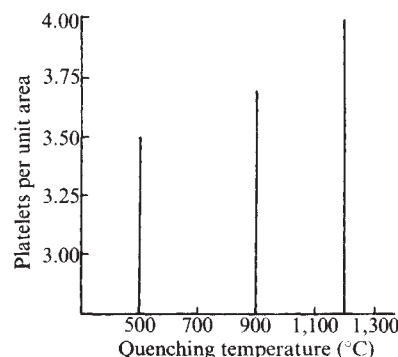


Fig. 2 Observed density of martensite platelets in samples quenched from various high temperatures.

temperature, with this time depending on the previous high temperature at which the sample is equilibrated (Fig. 1). As these data show, the higher the temperature from which the sample is quenched the shorter the time, t_0 , for the transformation to start. Also, as the transformation temperature is lowered, t_0 decreases.

In conjunction with the acoustic emission studies we have also measured the occurrence of martensite as a function of quench temperature at constant transformation temperature (Fig. 2). These data showed that, consistent with the acoustic emission data, the higher the temperature the more abundant the martensite. The density of martensite was obtained by standard optical microscope techniques, which involved counting the number of martensite platelets intersecting lines drawn on several optical micrographs—the standard method of assessing the density of a second phase.

Finally, in samples quenched from various temperatures, and held at the transformation temperatures for times $< t_0$, that is, where no acoustic emission signal was observed, we repeatedly observed no martensite platelets in the samples. This showed, under the ordinary definition of a transformation in this system, that no nucleation event had occurred, as once a nucleus is formed it is expected to grow. This proves that this procedure can distinguish between the nucleation event and the growth process in the martensite transformation.

An obvious interpretation of the present results, taking into account quenching studies in other metals and alloys, is that as we decrease the quenching temperature, we retain less vacancies. This can lead to fewer dislocation loops in those samples quenched from lower temperatures. Dislocation loops have already been suggested as possible nuclei for the transformation³, but very little has been done to exploit this approach. Small dislocation loops can provide a nucleus for the transformation, as loops, such as Shockley loops, can introduce regions of shear.

The present experiments show that there is a delay time in the transformation process and that this delay is affected by the temperature from which the sample is quenched. We have also shown that during this delay time no observable transformation occurs. A more quantitative and complete version of the present experiments will be reported elsewhere.

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A new geophysical tiltmeter

GEOPHYSICAL tiltmeters currently in use may be divided into two categories. The first are short baselength angle sensing systems, which have to be installed underground or only used above ground in ideal weather conditions. The second type are long-baseline hydrostatic levels and can be operated at, or just below ground level, but encounter problems with temperature variations. We describe here a new long-baseline tiltmeter for geophysical and engineering applications, it overcomes some of these problems and is capable of good performance when installed in a shallow trench.

The first type of geophysical tiltmeter, and by far the most widely used, is that of short baselength (between a few centimetres and a metre) angle sensing systems which include horizontal pendulums and more recently, electrolytic bubble levels. These instruments all suffer from the disadvantage that they are sensitive to short-wavelength temperature and rainfall generated tilting that is often much larger than the tidal or tectonic tilting which is to be observed. Such tiltmeters therefore need to be operated in controlled underground installations and are generally unsuitable for high sensitivity operation at ground level except under ideal weather conditions. Short-baseline tiltmeters are also more sensitive to signal generated noise in the form of strain generated tilts^{1,2}.

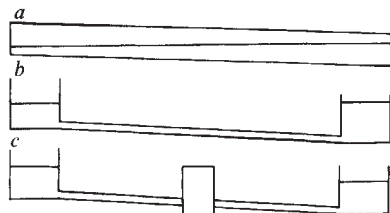


Fig. 1 *a*, Michelson's tiltmeter built in 1914, the tube was half full of water. *b*, Fluid level tiltmeter with tube filled with water. *c*, Differential fluid pressure tiltmeter.

The second class of instrument is the long-baselength hydrostatic level (between 10 m and 1 km). These measure the height difference between two points and are therefore relatively insensitive to short-wavelength weather generated tilting. Good results can be obtained with instruments installed at, or just below ground level. This was demonstrated by Michelson in 1914 (ref. 3) who obtained the first high quality Earth tide records using such an instrument. His tiltmeter, installed 6 ft underground consisted of a tube 500 ft long half-filled with water (Fig. 1*a*). He correctly appreciated that errors due to the thermal expansion of the fluid could be minimised by using a system with a large ratio of surface-area to fluid volume. Later long-baseline instruments have had the configuration shown in Fig. 1*b* presumably because this has been simpler to construct. However, unless the end pots have a very large area and the fluid volume is small, the system has a high temperature and pressure sensitivity. Unfortunately, this requirement directly conflicts with the need to have an instrument with a time constant of minutes rather than hours or even days. A short time constant makes setting-up easier and allows tides and rapid tectonic events to be observed.

There are two ways in which this problem may be overcome. One that has attracted some theoretical interest is to develop thermally compensated systems which permit the use of a small area to volume ratio without their inherent temperature problems^{4,5}. We have chosen to keep the area to volume ratio high. This seems a better approach than compensation because although a compensated system can in principle have a zero temperature coefficient, in practice this depends on an accurate knowledge and control of the thermal properties of the material involved and close control over the geometry of the instrument. We overcame the problem of long time constant by producing a system that does not depend on flow between the

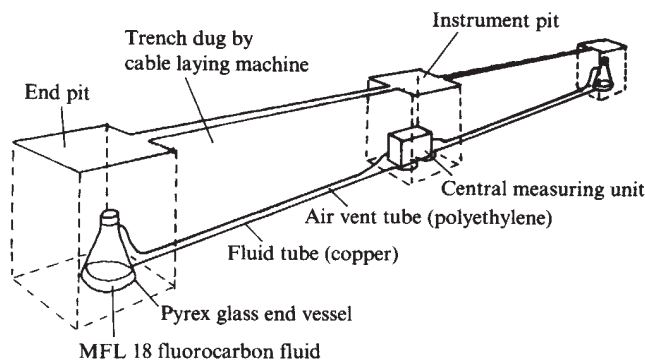


Fig. 2 The field prototype tiltmeter, 100 m long installed in a 1 m deep slot dug with a cable laying machine.

end vessels. It measures differential pressure at the centre (Fig. 1*c*) using a relatively stiff metal diaphragm with capacitive displacement sensing.

The measurement of pressure had other advantages. First, the measurement is performed at a single point at the centre, thus the end units are entirely passive. Second, because we are measuring pressure, the expansion of the fluid in the end vessels has no effect provided that the vessels themselves retain constant dimension. If the tube is approximately horizontal, temperature variations on the tube have negligible effect on the system because only a very small volume of fluid is contained in the tube. Thus the stability of the instrument depends almost entirely on the stability of the end vessels and the central measurement unit. A further advantage is that the instrument can be re-zeroed simply by opening a valve which connects the two arms of the instrument allowing the pressure to equalise.

Figure 2 shows field prototype tiltmeter. The end vessels of this instrument are 1 ft diameter pyrex glass laboratory flasks temporarily mounted in unconsolidated soil. The pipe is 4 mm i.d. copper tube laid in a 1 m deep 15 cm wide slot cut by a cable laying machine. The end pots are 100 m apart. A differential pressure resolution of 10^{-7} bar corresponds to a tilt of 5×10^{-9} rad. The fluid chosen is an inert fluorocarbon selected for high electrical resistivity, (necessary for the capacitive displacement measurement), resistance to bubble formation, low viscosity, and high density. The air space above the liquid in both vessels is connected by a 20 mm diameter polyethylene tube to equalise air pressure. An electronic system has been developed that consumes 500 μ A at 9 V permitting the device to operate from batteries.

Figure 3 shows the records from two parallel instruments. The tidal signal can be seen on parts of the original record and is clear in the power spectrum. Longer period signals could be due to volume changes in the soil caused by moisture transpiration by the long grass around the ends. Figure 4 shows a span of records from a period of settled dull weather which illustrates that the instruments are capable of tidal sensitivity. An earlier version of the tiltmeter installed in a disused railway tunnel had a stability better than 10^{-7} rad per month. We believe that the inherent stability of the instrument is 10 times better, and that better mounting methods than those used in our initial experiments are needed. We suspect that the differences between the traces in Fig. 3 represent initial settling of the end pots of tiltmeter 3 which were installed on loose sand immediately before the start of the displayed record.

The time constant of the 100 m instrument is ~ 1 min as opposed to ~ 4 h for an instrument of the same dimensions which measures fluid heights in the end pots, and it could

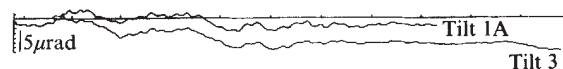


Fig. 3 45 d of record from two parallel tiltmeters. The time axis is divided at 100-h intervals.

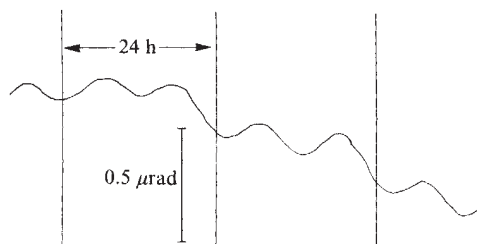


Fig. 4 72 h of record from one tiltmeter during dull weather. The data have been smoothed with a 1 h gaussian filter.

therefore be extended to 1 km or more without an unacceptable increase in response time and with a commensurate loss of sensitivity to mount instability.

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A new microfossil assemblage from the Archaean of Western Australia

THE oldest documented microfossils are from the ~3,300 Myr BP Onverwacht Group of South Africa^{6–8}. Here, we discuss the occurrence of a new assemblage of microfossils from a ~3,500 Myr BP silicified shallow-water to supratidal carbonate sequence of the Warrawoona Group at North Pole, Western Australia. Five morphologies of carbonaceous spheroids are recognised, including some with splits, some with tetragonal tetrad form and others with groups of up to four individuals. Their morphology is very similar to microfossils from the Onverwacht group of South Africa, and statistical tests of size distribution support a biogenic origin. This occurrence suggests that evidence for early Archaean life may be more widespread than generally thought.

The North Pole barite deposits (119°28' E; 21°07' S) are contained within the lower units of the Warrawoona Group of the Archaean Pilbara Block of Western Australia¹. The deposits occur 160 km SSE of Port Hedland, and 40 km W of Marble Bar. A concordant, bedded chert–barite unit is underlain by carbonated and silicified metavolcanic rocks, and overlain by similarly altered metabasalts. Metamorphism has been low grade and static. No isotopic age has yet been obtained from the North Pole barite deposits, but their stratigraphic equivalent, the Duffer Formation, has yielded ages between 3,450 and 3,490 Myr BP (refs 2, 3).

Beds within the chert unit display several primary sedimentary, and secondary diagenetic features, which are consistent with subtidal to supratidal environments of deposition⁴. Barite beds closely resemble some modern evaporitic sulphate beds (Fig. 1a, b), and some silicified clastic grains within chert beds show pseudomorphs after gypsum and anhydrite. Chert beds

show textures and fabrics similar to those of modern shallow-water to supratidal carbonate successions. These include silicified ooid-like grainstones, silicified intraclast and lithoclast grainstones, and silicified mudstones. In some localities, other textures within chert beds resemble those observed in stromatolitic carbonates. These include laminoid fenestrae, and a texture which resembles a stromatolitic edgewise conglomerate with pre- or syn-depositional oncolitic overgrowth (Fig. 2).

Samples which were thought to contain biogenic structures were macerated in HCl and HF using normal palynological preparation methods⁵. Organic matter was found in all the preparations, but in most of them, only amorphous reorganised carbonaceous matter was present. Two samples, however, (19h and 40) contained structurally preserved microfossils with a wide range of morphologies, including solitary spheroids, spheroids with splits, paired spheroids and rare chains of spheroids. Five of these morphologies will be briefly described here. All were observed in thin section with the exception of tetragonal tetrad forms which are rare in macerations.

The spheroids with splits are hollow (Fig. 3a, b), dark grey to black, spheroidal to ellipsoidal bodies. Their diameters range from 2.4 to 8.0 μm , with a mean of 4.4 μm . The surface of the wall is smooth, but the thickness is difficult to determine, although in general, it seems to be uniform and <0.5 μm . Each spheroid has one thinner, elongate area, which commonly appears to be a split in the wall. Several hundred specimens have been observed, and this morphology comprises 34% of the assemblage.

Smaller hollow, spheroidal dark grey bodies also occur (Fig. 3c). Their diameter ranges from 1.2 to 4.0 μm , with a mean of 2.0 μm . The wall surface is smooth and <0.5 μm thick. The bodies sometimes occur in pairs or fours, which may be enclosed within a common, almost transparent membrane. This

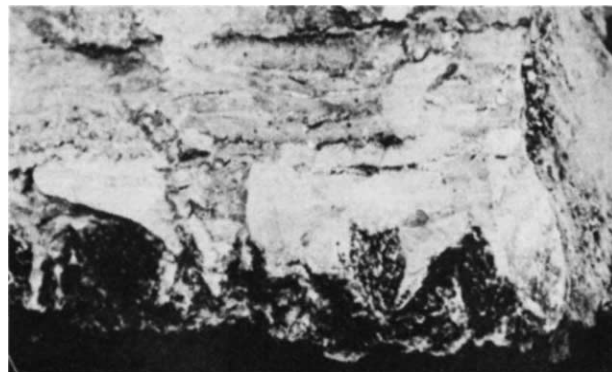


Fig. 1 North Pole barite showing sub-radiating crystal form. The crystal habit shows a striking resemblance to more recent gypsum occurrences. Width of field is 20 cm.



Fig. 2 Stromatolitic edgewise conglomerate from North Pole chert, showing oncolitic overgrowth on some fragments. The chert is secondary after original carbonate. Width of field is 1 cm.

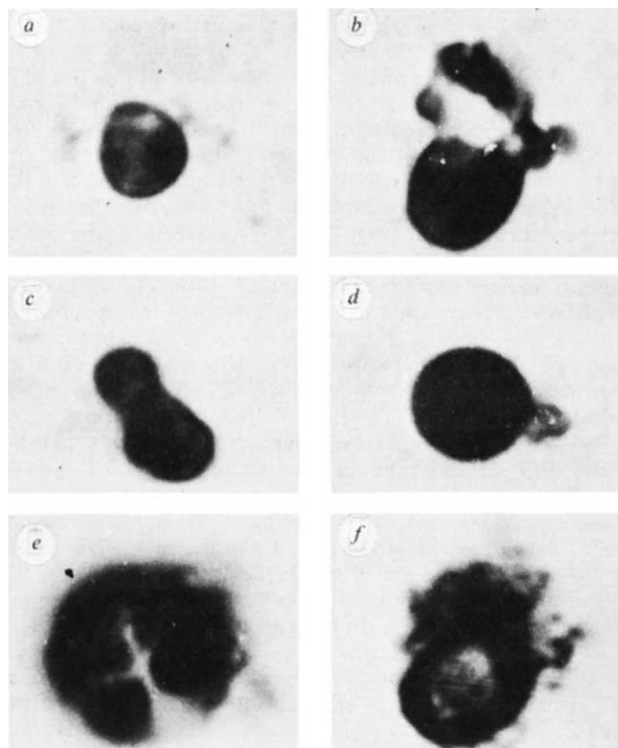


Fig. 3a Spheroid with split; **b**, spheroid with ruptured split; **c**, small spheroid without split, in a group of four; **d**, larger, hollow, black spheroid; **e**, tetragonal tetrad enclosed in almost transparent membrane; **f**, spheroid enclosed in dark amorphous organic matter. Width of field (**a**–**f**) is 10 μm . **a**–**e** come from sample 19h; and **f** from sample 40.

morphology comprises 47% of the assemblage, and several hundred specimens were observed.

Some larger, hollow, black spheroidal bodies are also present (Fig. 3d). A broken specimen of this type was found, and was clearly hollow. These appear to be thick-walled, simple spheroids, of relatively large diameter (8–12 μm), but their detailed morphology is difficult to determine because of their dense black colour. These spheroids are less abundant than either of the first two types.

A significant, but rare, morphology consists of an almost transparent spherical outer body with four dark coloured inner bodies, one in each quarter (Fig. 3e). Contact between the inner bodies is distinct, and the form of the whole is a tetragonal tetrad. The overall diameters of these bodies are between 7.0 and 8.0 μm , and that of the individual inner bodies is $\sim 3.0 \mu\text{m}$. Only two specimens have been discovered so far.

The last distinctive morphology consists of hollow, ellipsoidal bodies, embedded in amorphous organic matter, which may represent the remains of mucilaginous material (Fig. 3f). The maximum diameter is 4.0 μm , and the minimum 3 μm . The maximum diameters have an average of 3.5 μm (11 specimens). The wall is rough textured and thin ($< 0.5 \mu\text{m}$).

The morphologies of these spheroids are very similar to those of spheroids previously described from the Kromberg Formation of the Onverwacht group of South Africa^{6–8}, and from the Fig Tree Group from the same area⁹, although these latter assemblages are younger than the North Pole assemblage.

The diameter of the spheroids within the total population, and, separately, the diameter of the first two morphological types described above, have been statistically analysed, using size probability plot techniques^{8,10}. Monospecific biogenic assemblages with a narrow size distribution lie on a nearly straight, steeply inclined curve (like the extant algae *Chlorella* and *Gloeocapsa* (Fig. 4: data from Schopf¹⁰)). Heterogeneous biogenic assemblages plot along a curve which has several

breaks in slope (see ref. 8 for example). The North Pole total population gives rise to a curve with two distinctly different slopes, which may correspond to the two commonest microfossil types described above. However, these two distinctive types when plotted separately, have slopes that parallel those of the extant *Chlorella* and *Gloeocapsa*. The three separate bedding plane populations from the Onverwacht group Kromberg Formation described previously^{7,8}, also have similar slopes.

The probability graphs were plotted for two abiogenic populations of spheroids—these were synthetic quartz spheres, produced in silicification experiments¹¹, and some small silica-filled spheroidal structures which occur in a clast in one of the North Pole cherts. The structures are pale-coloured, and unlike any microfossils previously described. They do not have an organic wall, and their surfaces are picked out with a dusting of extremely fine-grained opaque minerals. However, many Precambrian microfossils are preserved by a coating of opaque minerals^{12–14}, so the inclusion of such spheroids in the same statistical treatment is not unreasonable. The size probability plot for these abiogenic spheroid populations is, however, quite different from the probability plots for *Chlorella*, *Gloeocapsa* and the North Pole spheroids.

Another way of determining whether the structures are biological in origin is the divisional dispersion index (DDI)¹⁰, which indicates the least number of vegetative divisions required mathematically to produce the smallest cell from the largest cell in a population. Populations of biological origin have DDIs generally well below 7 or 8, and commonly about 4. The DDI for the spheroids with splits is 6, that for the smaller spheroids also 6, and those for the abiogenic spheroids 8 and 7. By this criterion alone, they might possibly be regarded as showing biological characteristics, but their size probability plots are inconsistent with this interpretation. For the carbonaceous spheroids, however, their range of morphologies, their size distribution and their DDI, all suggest a biological origin.

This study, therefore, apparently confirms the presence of the remains of living organisms in ancient rocks of shallow-water origin. The statistical tests, together with the occurrence of spheroids with splits and rare tetragonal tetrad forms, support the interpretation of the spheroids as biogenic, and

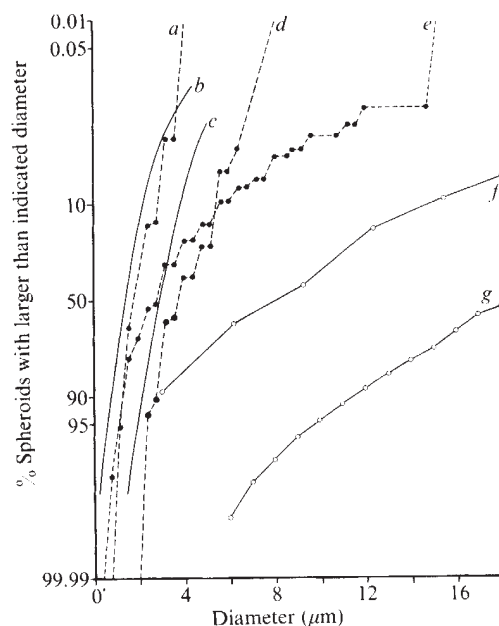


Fig. 4 Size probability plots for several populations of spheroids. The North Pole spheroids with splits are shown in Fig. 3a, b, and the small spheroids in Fig. 3c. **a**, Grey-black spheroids; **b**, *Chlorella* sp.; **c**, *Gloeocapsa* sp.; **d**, spheroids with splits; **e**, North Pole spheroids (total); **f**, North Pole abiogenic spheroids; **g**, synthetic quartz spheres.

further, the five morphologies described above show a sufficient morphological range to imply that even in these Archaean sediments, a considerable variety in the population exists. The assemblage is similar to that described from rocks of nearly equal antiquity⁶⁻⁹, and suggests that evidence for early Archaean life may be more widespread than was previously thought¹⁵. A comprehensive search for microfossils in other Archaean sediments in the east Pilbara Block is in progress.

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Very low salinity regions of estuaries: important sites for chemical and biological reactions

THE importance of biogeochemical interactions in estuaries is widely recognised^{1,2,4-6}; in particular, theoretical models of estuarine speciation of trace metals⁷ and of the pH-carbonate system⁸ predict that sharp changes of thermodynamic equilibrium conditions should occur at very low salinities (<1‰). However, because of the limitations of conventional sampling strategies¹², the chemical properties of this freshwater-seawater interphase (FSI) have not been adequately characterised. Instead, the expected variability has usually been represented by a scatter of spatially and temporally unresolved data points^{1,3,5,6}. Over the past two years, we have carried out periodic detailed investigations of the immediate mixing of the fresh and brackish water in the Tamar Estuary, South West England and we present data here for 11 determinands which point to the FSI as being an important site for chemical and biological processes in estuaries.

Interactive chemical processes involving the removal or addition of a dissolved constituent in estuarine waters have been inferred from nonlinear regressions of the dissolved constituent when plotted against a conservative index of mixing such as salinity or chlorinity^{1,2}. Nonlinearity may also be attributed to: (1) variabilities of flux and composition of the freshwater end-member over time scales comparable to the residence time of water within the estuary; and (2) subsidiary inputs of compositionally different waters. Therefore, the unequivocal demonstration of non-conservative (that is interactive) behaviour using salinity regressions requires a precise temporal and spatial characterisation of the end-members of the theoretical dilution line and thus necessitates careful and

frequent sampling at the seawards and, especially, at the freshwater ends of the estuary. The sampling strategy, described in detail elsewhere¹², is summarised in Figs 1 and 2. The inset chart in Fig. 1 shows the upper reaches of the Tamar Estuary and the positions of the 1.0‰ isohalines observed during four traverses of the estuary at different states of the tide on 17 and 18 August 1977. Oxygen supersaturation at the extreme seawards and freshwater ends of the estuary is a result of active primary production. The mid-estuarine O₂ deficiency is effected partly by the biological oxygen demand (BOD) of Plymouth sewage outfalls located at the mouth of the estuary (not shown on chart), and partly by increasing resuspension of bottom and intertidal sediments concomitant with approaching spring tide conditions. However, the most notable feature of Fig. 1 is the very sharp drop in O₂ which persistently occurs at the FSI, irrespective of the state of the tide or the position of the FSI along its ~13 km tidal excursion. It is unlikely that this enhanced O₂ depletion could have been generated by resuspension of anoxic bottom sediments because (1) it was absent in winter surveys, (2) resuspension, as measured by water column turbidity was found to be uniformly high (200–300 p.p.m.) and extended through the FSI and well into the fresh water, and (3) resuspension forces are not coupled to the salinity in the way the O₂ drop seems to be. In addition, there are no significant inputs of sewage in this area. Thus, the O₂ utilisation processes must be triggered in the water column at the FSI and are related to seasonal biological activity of the estuary.

Simultaneous profiles of O₂ and other chemical constituents obtained on 17 August are compared in Fig. 2. A stoichiometric balance of the oxygen deficit (ΔO_2) of 117 $\mu M O_2 l^{-1}$ generated in the 0.10–1.00‰ mixing segment against possible dissolved species which may undergo oxidation within this segment is shown in Table 1. The oxidisable inorganic constituents and the riverborne BOD entrained into the O₂ depletion zone together account for only 5.7% of ΔO_2 . If, however, the non-conservative decrease in the DOC peak (ΔDOC) centred at 0.2‰ is assumed to result from microbiological decom-

Fig. 1 Inset map of the upper reaches of the Tamar Estuary and the positions of the 1.0‰ isohalines during four traverses on 17 and 18 August 1977 (Plymouth City is located ~25 km downstream from Calstock). The corresponding dissolved O₂ concentrations as well as the 100% O₂-saturation profile are plotted against salinity.

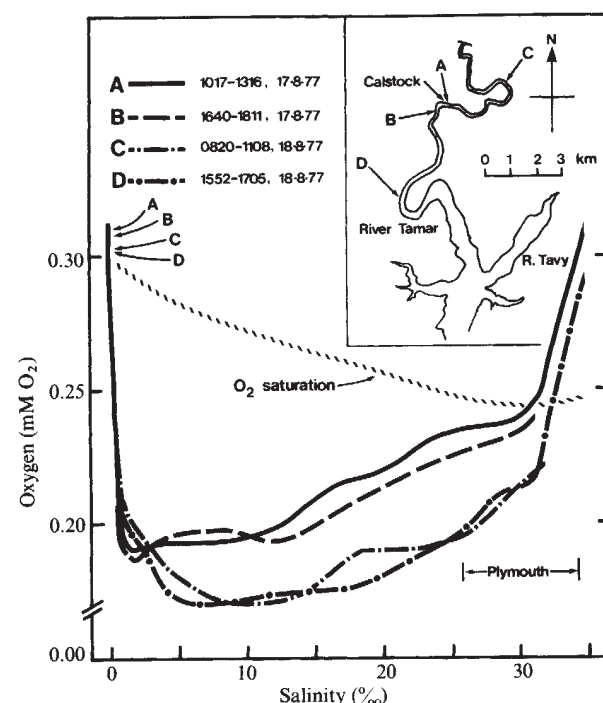


Table 1 Oxygen balance for the 0.10–1.00‰ mixing segment of the Tamar Estuary, 17 August 1977

Reduced species	Concentration in R. Tamar (μM)	Oxidised form	O ₂ requirement (μM)	% of ΔO_2 ($\Delta\text{O}_2 = 117 \mu\text{M}$)
NO_2^-	1.0	NO_3^-	0.5	5.4%
NH_3^*	2.1	NO_3^-	4.2	
Fe(II)^*	2.3	Fe(III)	0.6	
Mn(II)	2.0	Mn(IV)	1.0	
$\Delta \text{DOC-C}$	102	CO_2	81.6	69.7%
$\Delta \text{DON-N}$	15.4	NO_3^-	24.6	21.0%
BOD^*	44.0	CO_2	0.4	0.3%

ΔO_2 corresponds to the decrease in dissolved oxygen between fresh water and the 1.00‰ isohaline, as shown in Fig. 2. Maximum O₂ requirement for inorganic oxidation reactions were estimated by assuming all the dissolved Fe and Mn to be in the (+II) oxidation state, and that the oxidation product of NH_3 and NO_2^- is NO_3^- . ΔDOC is the non-conservative decrease of the DOC peak (see Fig. 2) by microbiological decomposition to CO_2 , whereas ΔDON is the associated organic nitrogen estimated using N:C ratio of 16:106¹⁴. The mineralisation of organic matter to CO_2 and NO_3^- was assumed to be 80% efficient²⁶. BOD 'concentration' is equivalent to the quantity of O₂ used in a 5 day incubation at 20 °C¹⁶. The corresponding O₂ used within the 0.10–1.00‰ mixing segment was calculated from the mean residence time $\bar{R}$ of 9.3 h for this segment. ($\bar{R} = \bar{F}/\bar{V}$; $\bar{F}$ (mean river flow at Gunnislake, 17 August 1977) = $3.096 \text{ m}^3 \text{ s}^{-1}$; $\bar{V}$ (volume of 0.10–1.00‰ segment of the estuary at 1300, 17 August 1977) = $1.04 \times 10^5 \text{ m}^3$.) O₂ production by photosynthesis and reaeration over time period $\bar{R}$ was estimated to be <2% of ΔO_2 , and thus omitted from the calculations.

* Data obtained on 18 August from B. Milford, South West Water Authority.

position of the dissolved organic matter to CO_2 and NO_3^- , then it is possible to account for a maximum of ~91% of ΔO_2 (see Table 1 for details). The coincidence of the O₂ sag and the DOC peak with the chlorophyll fluorescence minimum within the 0.10–1.00‰ mixing segment suggests that they are coupled by a common mechanism. We hypothesise this mechanism to be a sequence of processes starting with mass mortality of fresh water halophobous phytoplankton incapable of withstanding the sharp sudden osmotic and compositional changes of the FSI. This results in a continuous plasmolytic release of easily degradable dissolved organic material, which, in turn, supports a localised, but active, population of O₂-utilising bacteria.

Applying this 'osmotic hypothesis' to our data, it follows that the release of ~100 μM DOC-C l^{-1} at the FSI would be brought about by the plasmolysis of a biomass of fresh water phytoplankton containing 40 μg chlorophyll-*a* l^{-1} (C: Chl-*a* = 60:1 (ref. 21); soluble cell contents = 50%). Comparable or

greater levels of chlorophyll-*a* are encountered in productive rivers²⁸. However, apart from isolated taxonomic observations^{19,20} pointing to the absence of freshwater species of phytoplankton in estuarine and marine waters, there do not seem to have been any quantitative investigations of the fate of riverborne phytoplankton in estuaries. In steady-state conditions the 0.10–1.00‰ segment, which can be shown to be wholly contained within the second mixing compartment of a Ketchum-type estuarine tidal circulation model²², is characterised by a hydrodynamic exchange ratio (*r*) equal to 0.89. The rate of reproduction necessary to maintain a constant

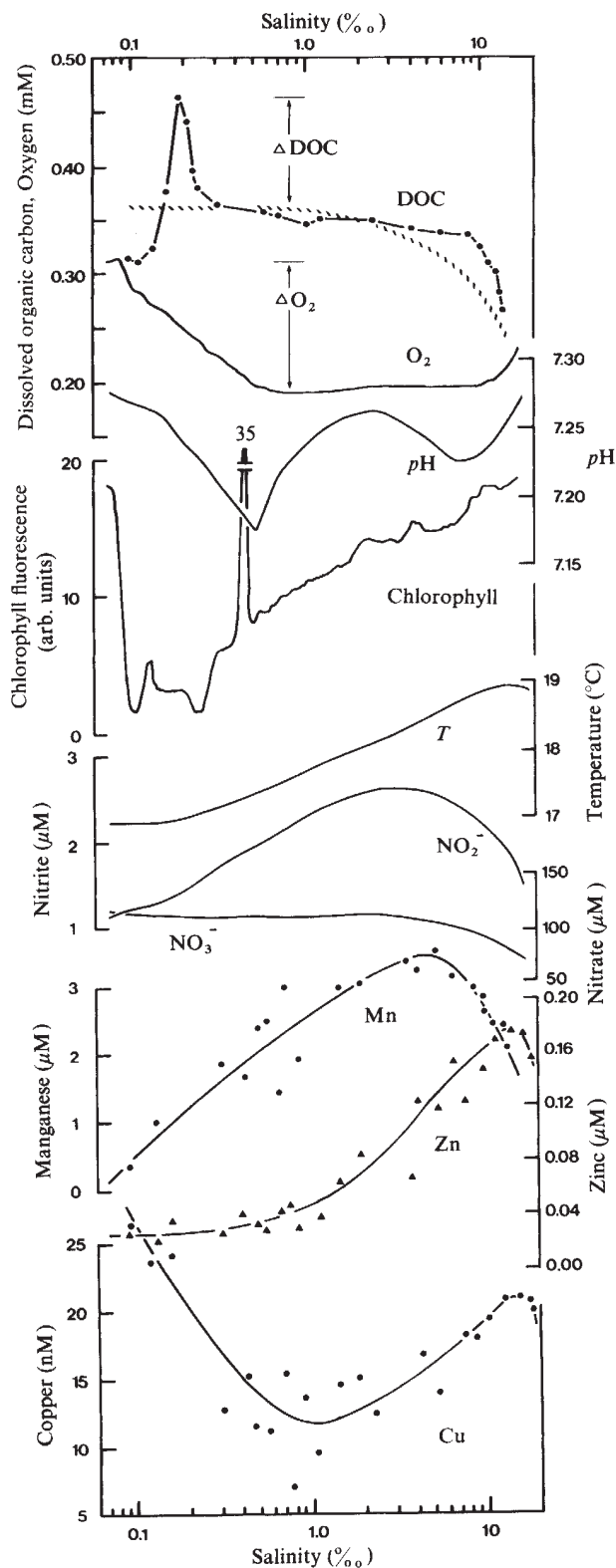


Fig. 2 The estuarine profiles for O₂, pH, relative chlorophyll fluorescence, temperature and the concentrations of dissolved organic carbon DOC, conservative DOC profile (---), nitrate NO_3^- , nitrite NO_2^- , manganese, zinc, and copper obtained on 17 August 1977. These have been plotted against $\log_{10}$ salinity (‰) to resolve spatially the concentration changes which occur at low salinities. In conjunction with an Electronic Switchgear MC-5 salinity-temperature bridge, a chloride selective electrode (Phillips 1S 550-Cl) calibrated against standards of known salinity was used for indicating the degree of mixing in regions of low salinities. Continuous records of Cl^- electrode response, O₂ concentration (Model 57, Yellow Springs Instruments), pH (Pye-Ingold Model 400 series) and relative chlorophyll fluorescence (Turner Instruments) were obtained during the traverse. DOC in GF/C glass fibre filtered samples was determined in triplicate using a modified sea-going version of an automated photochemical analyser¹⁷. NO_2^- and NO_3^- were determined using conventional Technicon Autoanalyser^(R) coupled to a continuous filtration system¹³. Mn, Zn and Cu were determined by preconcentration of membrane filtered samples on chelating resin (NH_4^+ form) followed by atomic absorption spectrophotometry¹⁸. Recoveries and calibrations for all determinands were checked using standard additions to samples of various salinities.

endemic population of bacteria in this segment in spite of continuous depletion by river run-off and tidal circulation may be derived from the equation²³ $k = -\ln(1-r)/12.4$ where k is the specific reproduction rate and is equal to 0.18 h^{-1} . The corresponding division time of 3.85 h is a measure of the microbiological activity required to maintain the sharp O_2 depletions shown in Fig. 1. It follows that the 'chemostatic' decomposition of ΔDOC shown in Fig. 2 would require an active population of $1-4 \times 10^6 \text{ cells ml}^{-1}$ (1 bacterium = 10^{-13} g C (ref. 25); growth efficiency = 0.25–0.75 (refs 26, 27 respectively)) which compares favourably with direct bacterial enumerations reported for the brackish regions of Kiel Fjord²⁴.

The sharp increase in $[\text{NO}_2^-]$ starting from $1.0 \mu\text{M NO}_2^- \text{ l}^{-1}$ at the FSI and reaching a maximum of $2.7 \mu\text{M NO}_2^- \text{ l}^{-1}$ at 3.1% represents an intermediate stage in the oxidation of DON and NH_3 to NO_3^- . However, it is difficult to observe the subsequent production of NO_3^- as a positive deviation from the theoretical dilution line because analytical precision would have to be better than 1% for the very abundant background levels of NO_3^- ($\sim 120 \mu\text{M NO}_3^- \text{ l}^{-1}$) in the Tamar River.

The pH minimum of 7.18 centred at 0.5% is partly due to the mineralisation of DOC and partly to the sharp changes in the conditional stability constants of carbonic acid resulting from increasing ionic strength across the FSI^{8,11}. Such perturbations in pH of estuarine waters have a profound effect, not only on the adsorption equilibria of trace metals with suspended particulate material⁹ but also on their speciation and reactivity^{7,10,15}. The Mn, Zn and Cu profiles in Fig. 2 are non-conservative. The mid-estuarine maxima common to all three metals and to water temperature correspond to a natural input of metal-rich solar-warmed pore water from the large areas of intertidal mudflats in the mid-estuarine regions of the Tamar. However, the estuarine chemistries of Mn, Zn and Cu within the 0.10–1.00% mixing segment are conspicuously different. It is difficult to quantify and identify which of the many potential parameters and reactions (such as, pH, S° , redox and adsorption equilibria, and kinetic barriers^{9,10}) could account for the observed differences at the interphase.

The fivefold increase in Mn is surprising when one considers its well established interactive nature with Fe^{11} , which is a highly non-conservative constituent of estuaries^{1,2}. Preliminary experimental studies of Mn in the Tamar Estuary strongly suggest that the overriding process is a kinetically controlled heterogeneous redox mechanism, displaying a salinity dependence. In contrast, the electrochemically more inert Zn is conservative within the 0.10–1.00% mixing segment. A negative Cu slope preceding the mid-estuarine increase may indicate either removal of Cu from solution or, more likely, a change in Cu speciation into an analytically unavailable form (such as, Cu-organic complexes)¹¹.

Clearly, the FSI is a chemically and biologically interactive zone requiring greater attention and characterisation than has been undertaken in previous estuarine investigations. The development of more precise geochemical cycling models and the refinement of estuarine oxygen models used for management purposes are two important topics which would benefit considerably from a fuller understanding of these interactions occurring across the FSI.

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Information transmission in remote viewing experiments

TARG AND PUTHOFF^{1–3} have described investigations of an extrasensory remote viewing ability which they claim may be widely distributed in the general population. We have carried out duplicate experiments, but our results do not verify their conclusions.

In their experiments on remote viewing of natural targets with Pat Price¹, and later with other subjects^{2,3}, the subject remained with an experimenter at Stanford Research Institute (SRI) and at a prearranged time gave a description of an undisclosed, remote site being visited by a team of two to four co-experimenters. Each of nine remote locations was selected from over 100 targets within a 30-min drive of the laboratory. At the end of each trial the subject was taken to the target site to provide feedback.

To evaluate the accuracy of the remote viewings, the descriptions and a list of the nine targets were presented to independent judges who visited each target location in turn. Each judge chose the best description from the set of nine at each location. Five judges produced a total of 24 correct matches for the Price series ($P = 8 \times 10^{-10}$). In a further judging procedure, another judge, Dr Arthur Hastings, was asked to rank each of the nine transcripts on a scale from 1 to 9 (best to worst match) against each target location. The sum of ranks assigned to the target-associated transcripts was obtained. For nine targets this sum can range from nine (for a perfect matching) to 81, chance being 45. The judge produced a sum of ranks of 16 for Price ($P = 2.9 \times 10^{-5}$)⁴.

During a recent visit to SRI D.M. attempted to duplicate the ranking procedure using transcripts from the original experiments with Price reported by Targ and Puthoff^{1–3}. After discussions with Dr Hastings, the Price series of transcripts was made available for examination and it was agreed that I (D.M.) should rank in order a subset of five transcripts against five target locations. The remaining four locations and transcripts

were excluded as they had already been published and I would have seen their pairings. Although not stated in the original reports the judges were provided with a listing of targets in the correct (original) sequence. In addition, careful examination of the transcripts indicated that a large number of cues were available indicating the position of a transcript in the series; for example: (1) Price expresses apprehension and an inability to do this kind of experiment (Target 1); (2) a reference is made to the fact that this experiment is the "second place of the day" (Target 2); (3) a reference is made to "yesterday's two targets" (Target 3); (4) Targ says encouragingly: "Nothing like having three successes behind you" and mentions the nature reserve visited the day before (Target 4); (5) Price refers to the Marina which was the fourth target (Target 7). Using these, and other, cues I was able to match the five transcripts correctly with a rank of one, giving a sum of ranks of five ($P < 0.0005$) (ref. 4). In this procedure it should be noted that I had never visited any of the five locations but completed the task solely on the basis of cues contained in the transcripts.

In a further experiment we determined whether identification of target sites could be made from the transcripts without using the extraneous cues. To ensure that judges were blind to the correct matchings it was again necessary to exclude the four transcripts already published. The remaining five locations, all in the Stanford, California area, were Baylands Nature Preserve, the Radio Telescope in Stanford Hills, the Toll Plaza at Dumbarton Bridge, the old Drive-in Theatre at Palo Alto, and the Church of Our Lady of the Wayside on Portola Road. Two judges, both research psychologists, visited these targets in a random sequence and independently ranked the five transcripts at each location, extraneous cues having been removed.

Each judge made a detailed content analysis of the descriptive information provided in the transcripts and ranked them on the basis of the descriptive accuracy of the information available. The two judges produced sums of ranks of 16 and 13, neither of which departs significantly from the chance expectation of 15.

These results provide a striking contrast to the correct matchings obtained when the cues from the unedited transcripts were used in making the judgments. The null result also contrasts with the significant matching obtained with unedited transcripts by the original SRI judges.

With cues as strong as, 'Nothing like having three successes behind you', (Transcript 4) it is difficult to imagine that judges could overlook or not be influenced by them, and yet the judges repeatedly considered themselves 'blind'. One explanation is that the judges used the extraneous cues to form hypotheses which were then subjectively validated in the transcript proper. In our work with remote viewing we have observed the occurrence of strong subjective validations, a selective awareness of similarities between the subject's description and the target location, once the correct match was revealed, even though the same transcripts produced random matches when judged blind. Of course, the illusion of a good match can be magnified in published reports by the use of *post hoc* photographs of the location, and by selected excerpts from the transcripts.

Our investigation of the SRI remote viewing experiments forces the conclusion that the successful identification of target sites by judges is impossible unless multiple extraneous cues which were available in the original unedited transcripts are used. Investigators of remote viewing should in future take care to ensure that such cues are not available. Furthermore, the listing of targets given to judges should be randomised and not presented in the same sequence as that which occurred in the experiments.

Until remote viewing can be confirmed in conditions which prevent sensory cueing the conclusions of Targ and Puthoff remain an unsubstantiated hypothesis. Our own experiments on remote viewing under cue-free conditions have consistently failed to replicate the effect.

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Continuity and versatility in bird song: support for the monotony-threshold hypothesis

HARTSHORNE^{1,2} has proposed that a direct relationship exists between the degree of continuity and versatility in bird song. In other words, the more continuously a bird species sings (that is, the longer the song lengths relative to pauses between songs), the higher is the probability that successive songs will be different. Birds of a given species may sing with immediate variety, where successive songs are different, whereas other species tend to sing with eventual variety, where a song type is repeated many times before another is introduced; males of some species have only a single song type, and thus show a lack of variety in their singing. Hartshorne proposed that a monotony threshold exists, making singing behaviours with high continuity and low versatility too monotonous and therefore ineffective. Because Hartshorne collected information primarily by ear, his data on singing behaviours were not always accurate³; also, his philosophical approach to the aesthetics of bird song remains incompatible with the more rigorous approaches used by most biologists. His hypothesis has been further discredited by a report⁴ which, through quantitative analyses of data compiled for 39 species, found no significant correlation ($r_s = 0.20$) between versatility and continuity of singing. However, because Dobson and Lemon⁴ used repertoire size as the measure of versatility, Hartshorne's monotony-threshold hypothesis was not actually tested, for his^{1,2} measure of versatility involved the sequential organisation of different songs during a singing session, not merely the total repertoire size. This is an important distinction, as Hartshorne^{5,23} stresses, for a species such as the Carolina wren (*Thryothorus ludovicianus*) may have a large repertoire size but sing a song type 100 times before changing to another. Hartshorne considers such song patterning to be non-versatile^{5,23}, whereas Dobson and Lemon rank this species as highly versatile. The purpose of this report, then, is threefold. First, because of these differences in the definition of versatility, I test Hartshorne's monotony-threshold hypothesis, using the original and some revised (see Table 1) data tabulated by Dobson and Lemon⁴. Second, I discuss the difficulties involved in measuring continuity and versatility among species with very different singing behaviours. Finally, I review data from wrens (Troglodytidae) which reveal a clear relationship between several different and, I feel, improved measures of continuity and versatility.

First, if Hartshorne's predictions are true, species singing with immediate variety will have a higher percentage performance time than will species singing with only eventual or no variety. Overall, there is no significant difference among those species tabulated by Dobson and Lemon (1-tailed Mann-Whitney *U*-test, $P > 0.1$). However, close inspection of the data reveals that the nine *Vireo* species are unique in that males sing very short songs many times a minute; these vireos differ significantly from the other 30 species with respect to song length, inter-song interval, and consequently, percentage performance time ($P < 0.001$ in all three measures, 2-tailed

Mann-Whitney U test). If the non-vireo species are examined separately, those species tending to sing with immediate variety do sing with greater continuity than do the less versatile songsters ($P < 0.001$, 1-tailed Mann-Whitney U test); thus, Hartshorne's proposed hypothesis is supported by the non-vireo data.

Hartshorne's hypothesis was concerned primarily with interspecific differences in continuity and versatility, but these cross-species comparisons involve several problems, not the least of which is the definition of a 'song'. The discrete units uttered by many sparrows, for example, are quite different from the continuous rambling of a mockingbird (*Mimus polyglottos*) or an American robin (*Turdus migratorius*)⁴. The degree of contrast between successive songs³ also varies considerably among species, and the complexity within some longer songs of non-versatile songsters may provide enough signal contrast to prevent monotony; it is perhaps significant that song lengths (= song complexity?) are inversely related to versatility, for species singing with no, eventual, and immediate variety have median song lengths of 2.16, 1.66 and 1.02 s, respectively. Also, percentage performance time may not be a very good measure of continuity, as it seems highly dependent on song length. Overall, the vireos have brief songs and a correspondingly low percentage performance time; among the vireos, species 30 has the shortest song and the lowest percentage performance time, whereas species 22 has the longest song and the highest percentage performance time. Similar extremes also occur among finches and wrens, for the large-footed finch (*Pezopetes capitalis*) and the winter wren (*Troglodytes troglodytes*) have average song lengths of 0.7 and 6.3 s and performance times of 9 and 50%, respectively. Contrary to what Hartshorne would have predicted from such continuity measures, the finch sings with immediate variety whereas the wren sings with eventual variety (ref. 6 and D.E.K., unpublished data). Finally, in those species where males have large repertoires, eventual or immediate variety in song patterning may be used in different contexts (see below).

Most of the above complications introduced by inter-species differences in singing behaviours can be avoided by studying

closely related species, different individuals or populations of the same species, or the same individual at different times of the day or nesting cycle. Thus, work with rock (*Salpinctes obsoletus*) and marsh (*Cistothorus palustris*) wrens has revealed that time intervals between songs of the same song type are longer than are intervals between songs of different song types^{3,7}; this is consistent with the continuity-versatility hypothesis. The sedge wren (*Cistothorus platensis*) is also ideal for detailed study, for males may have over 100 song types and sing with either immediate or eventual variety, depending on the motivational state of the individual.

For the sedge wren, three measures called song type versatility, transition versatility and total versatility are more useful than the discrete categories of versatility proposed by Hartshorne. Song type versatility is the number of different song types in a sequence, and transition versatility is the number of transitions which are between unlike songs. In a sequence of 10 songs, song type versatility can range from 1 to 10, and transition versatility from 0 to 10. Total versatility is the product of song type and transition versatility, and its value ranges from 0 to 100. Detailed analyses of the singing of three individual sedge wrens revealed a high correlation between the percentage performance time and all three measures of versatility ($P < 0.05$), but the correlation of song rates with the versatility measures was even higher⁸.

Thus, a key factor influencing versatility may be the number of song stimuli delivered per unit time. Indeed, Hartshorne's overestimates for continuity of singing in vireos⁴ reveal that his values were heavily influenced by the high song rates. Examination of Table 1 reveals that species singing with no, eventual, or immediate variety have median song rates of 5.9, 7.6 and 19.6 songs per min, respectively, and that species singing with immediate variety deliver more songs per unit time than do non-versatile songster ($P < 0.001$, 1-tailed Mann-Whitney U test). (The trend persists even with vireos removed from the data, with song rates for no, eventual, and immediate variety being 5.6, 7.6 and 11.2 songs per min, respectively.)

Habituation during vocal interactions may play an important part in the population biology of songbirds, and is probably

Table 1 Song data for various species of birds

Species	Repertoire size	Song length	Intersong interval	% Performance time	Ref.
10*†	16 (3)	1.71 (2.39)	4.56 (6.73)	29 (22)	14–16 (15, 16)
11‡§			6.23 (4.48)	22 (35)	17 and D.E.K., unpublished data (16, 18)
16¶¶			10.4 (11.60)	13 (11)	19(20)
21**	0.33 (0.35)	0.33 (0.35)	2.04 (0.98)	33 (51)	
22††			1.58 (1.57)	24 (25)	
25*†			3.71 (6.98)	30 (19)	14–16 (15, 16)
26‡‡	0.35 (0.33)	0.35 (0.33)	6.07 (9.18)	30 (22)	21, 22 (14, 22)
27§			1.30 (1.29)	11 (12)	
28††‡‡			2.25 (2.47)	15 (14)	
30††					
31††§§					

These results are values revised from Table 2 of Dobson and Lemon⁴; original entries are in parentheses and justifications for revisions are made in footnotes. Briefly, revisions for species 7 (omitted, see §§), 11, 16, 21 and 27 are, I feel, major improvements, whereas revisions for species 22, 26, 28, 30 and 31 are relatively trivial. Revisions for species 10 and 25 are based on my opinion that the singing rates of Reynard¹⁴ are preferable to the average of the range of singing rates given by Borror¹⁶.

* Original entries calculated from singing rate of '6–8 songs per min'¹⁶, using a mean of 7 songs per min.

† Preferred values based on cadence of Reynard¹⁴.

‡ Revised values based on larger samples.

§ Original entries from a combination of east and west coast populations of North America; all revised values are from one west coast population.

¶ Original entries were from a single male, revised values are population averages.

¶¶ Data are not in the ref. given; this is also true for species 6, 20, 23 and 29 which are not relisted here.

** Original entries were based on maximum singing rate; revised values are based on population averages.

†† Rounding errors in original entries.

‡‡ Song lengths for Eastern and Western populations of solitary vireo (species 26 and 28) had been reversed.

§§ In addition to revisions indicated here, species 7 was deleted from the table because data were based on $n = 1$ intersong interval. According to the above refs and in the original table of Dobson and Lemon, species 1–6 were considered non-versatile, species 8, 9, 11, 16, 17, 20, 23, 27 and 33 were eventually versatile, and species 12–14, 18, 21, 22, 24–26, 28–31 and 34–39 normally sing with immediate variety.

involved (1) in the process of neighbour 'recognition' among territorial males (for example, ref. 9), (2) in the defence of territories against intruder males¹⁰, and (3) in the evolution of large repertoires of song types which may be used in territorial interactions in dense populations⁶ or in the attraction or stimulation of females during the mating process¹¹⁻¹³. The correlation between continuity and versatility in data discussed in this report supports the hypothesis that the threat of habituation (that is, monotony) has influenced the sequential and temporal patterning of signal variety during vocal display.

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Deep croaks and fighting assessment in toads *Bufo bufo*

ANIMALS often settle disputes by means of conventional displays. It has been suggested that this enables the contestants to assess each other's strength without resorting to a serious fight¹. If this is true then we would expect natural selection to favour displays which give reliable information about fighting potential; assessment signals that are easily mimicked by weak individuals will not be evolutionarily stable^{2,3}. Often the outcome of a contest will depend simply on who is the larger, and many displays seem to involve assessment of body size^{1,4}. In this paper we show experimentally that male toads, *Bufo bufo*, settle contests for the possession of females by means of vocalisations that give a reliable signal of body size and hence of fighting ability.

Toads spend most of the year on land but each spring they mass together in ponds to breed, all the spawning taking place within a week or two. The male clasps on to the female's back (amplexus) and may be carried around by her for several days before she eventually lays her eggs, whereupon he fertilises them externally. Our field observations were made in 1977 and 1978 at a pond near Oxford where we marked several hundred toads individually with numbered elastic waist bands⁵ or by unique combinations of toe-clipping.

The toads arrived at the pond by night and 84.4% ($n = 77$) of the females that we found on land, on their way to the water, already had a male riding on their back. In the pond itself, all the females that we saw were paired ($n = 150$). Although all the females acquired mates, only a quarter of the males did so. The excess of males came about partly because males spent all the breeding season in the pond, some mating more than once, whereas the females visited the pond for a shorter time. Thus,

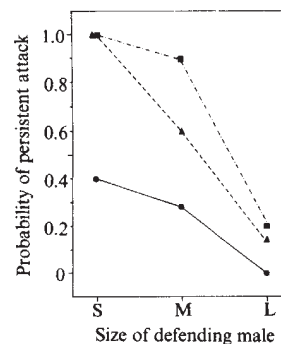


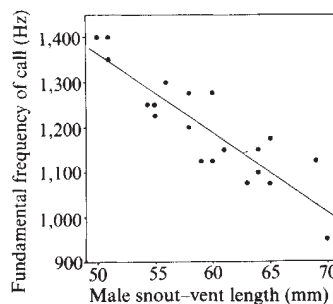
Fig. 1 Single male toads are more likely to persistently attack paired males who are smaller than themselves. A persistent attack is defined as repeated attempts by the single male to dislodge the paired male, involving attachment to the pair and prolonged struggles. Each point is based on 12 laboratory experiments in which a single male was placed in a tank together with a pair. Size classes of male toads: ●, small (50-54 mm snout-vent length); ▲, medium (56-60 mm); ■, large (62-66 mm).

on any one day all the male population was present but only a fraction of the female population. However, this is not the entire story because even over the whole breeding season there were still more males than females.

The presence of all these unpaired males gave rise to intense competition for mates and we often saw two or more males tussling for the possession of a female both on the land and round the spawning sites in the pond. Single males attempted to dislodge paired males by pushing in between their rival and the female, either from the front or the back. The paired male sometimes managed to kick the attacker away, but if a single male got a hold a vigorous struggle ensued, which could last several hours as the attacker strove to achieve a takeover. Twenty out of 52 marked males (38.5%) were displaced from females in this manner before the female spawned. Males varied in size from 49-70 mm snout-vent length, probably largely a reflection of their different ages. In 11 out of the 14 cases in which the sizes of the competing males were known, the takeover was by a larger male. Similarly, in fights in the laboratory we found that large males could sometimes displace smaller ones (10 out of 23 cases) but not *vice versa* (none out of 18 cases)⁶.

Because body size was such a good predictor of fighting success, we were interested to see whether a single male would ever attempt to engage in a long struggle with a paired male who was larger than himself. In the pond, 11 out of the 13 persistent fights that we observed between unmarked males were cases in which an attacker was attempting to dislodge a smaller male. In the laboratory we also found that males were only likely to persistently attack rivals who were smaller than themselves (Fig. 1). When the paired male was larger, the spare male usually gave up his attack after a brief encounter.

Fig. 2 Fundamental frequency of a defending male's call is closely related to his body size; larger toads make deeper pitched croaks ($\hat{y} = 2258.75 - 17.83x$, $r = -0.884$, $P < 0.001$). All of the 20 toads were recorded in the laboratory at 15°C and the fundamental frequency was measured from sonograms.



What cues does the attacking male use to assess the size, and thus fighting ability, of his rival? Whenever a male attacked a pair, the defending male always called ($n = 156$). From sonograms, the structure of this call seems to be identical to that of the 'release call', which is given by a single male whenever he is clasped mistakenly by another male in search of a female^{7,8}. Because the attacker hears the same call in the two contexts, he obviously cannot use it to inform him as to whether the caller is single or paired. Some other cues must be used because an attacker always immediately releases his grip of a single male but may persistently fight with a paired male.

However, once the attacker has detected a pair, he may use the call of the defender to influence his decision whether to attack and attempt a takeover. As reported for other anurans⁹⁻¹², the frequency of the call is closely related to body size; larger males have deeper pitched croaks (Fig. 2). The fundamental frequency of the call is determined by the mass, length and tension of the vocal cords^{8,13}. Larger individuals have a larger larynx¹⁴ and are therefore able to make a deeper croak. Thus, because call frequency is anatomically constrained, it could potentially give an attacker reliable information as to the size of his adversary.

To test this hypothesis we collected 24 medium-sized males (56–60 mm snout–vent length) from the pond and carried out an experiment in the laboratory. Twelve of these males were used as attackers against small paired males (50–54 mm) and the other 12 were used as attackers against large paired males (62–66 mm). The paired males were silenced by fitting them with small rubber bands placed behind their arms and passing through their mouths like a horse's bit. This prevented them from croaking, but it did not seem to affect their behaviour in any other way; they still gripped the female tightly in amplexus and kicked out vigorously at any attacker. Females of the same size were used in all the trials and no pairs were used for more than three attackers.

Each attacker was used in two experiments with the same silenced, paired male. In both trials he was placed in a small tank, and above the paired male we fixed a small loudspeaker through which we played tape-recorded croaks of either a small male (high pitched croaks) or of a large male (deep croaks). Previously we had found no significant relationship between body size and the rate of calling ($r = 0.084$, $n = 19$) nor the amplitude of the calls ($r = 0.401$, $n = 22$), so the croaks were broadcast at the same rate and at the same amplitude in each trial, the only difference being the pitch of the call. Each experiment was carried out at about 15°C and lasted 30 min. Whenever the spare male touched the pair, we broadcast croaks for 5 s and recorded the attacker's behaviour. The size of the tank restricted the movement of the pair, who usually remained quite still and did not behave differently between treatments. Half the attackers heard the high pitched croaks first and half heard the deep croaks first, with about 3 h between each trial.

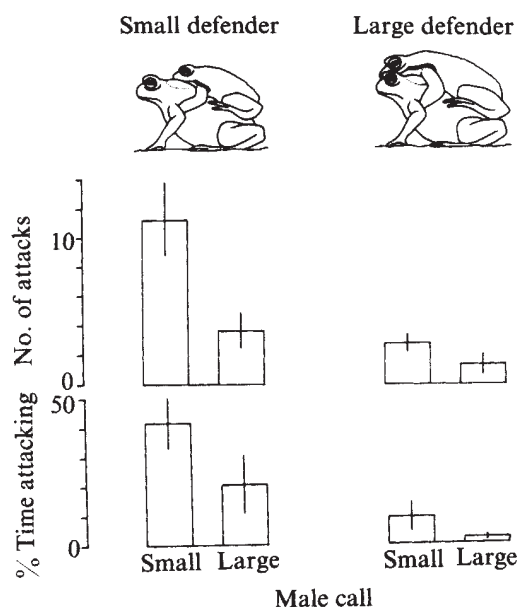


Fig. 3 Medium-sized males were given the opportunity to attack small or large paired males who were prevented from croaking by rubber bands passing through their mouths. Each attacker was used in two experiments. In one, he heard tape-recorded croaks of a small male and in the other the croaks of a large male. One set of 12 males attacked small defenders and another 12 attacked large defenders. Two measures of attacking behaviour were used, the number of attacks and the percentage time attacking. Histograms represent mean scores, with vertical lines indicating s.e.m. See also Table 1.

The results show that an attacker's behaviour can be manipulated by the frequency of the call that we broadcast to him (Fig. 3, Table 1). He was much more likely to attack the paired male when we played a high pitched croak than when we played a deep croak. If the call was the only cue that influenced the attacker's behaviour, then we would predict that when the broadcast call was the same frequency, small and large defenders would be attacked with equal vigour. However, fewer attacks were delivered to the large paired males. So although the playing of a deep croak inhibited attacks against small defenders, we could not induce a male to persistently attack a large defender by broadcasting high pitched croaks. Other cues, such as tactile cues or the strength of the defender's kick presumably gave the attacker information as to the real size of his adversary.

The advantage of calling to a large defender is clear; his deep croaks give other males a reliable indication of his body size and deter potential attackers. The signal is reliable in the sense that the pitch of a large male's croak is resistant to bluff by a

Table 1 Analysis of variance of the results summarised in Fig. 3

Source of variation	Degrees of freedom	No. of attacks		% Time attacking (arcsine square root transform, radians)	
		Mean square	Variance ratio	Mean square	Variance ratio
Between small and large defenders	1	346.69	10.4†	0.1847	13.1†
Within small or large defenders	22	33.27	—	0.0141	—
Total defenders	23				
Between call type	1	247.52	11.8†	0.1910	20.7‡
Interaction, defender size and call type	1	111.02	5.3*	0.0186	2.0
Residual	22	21.04	—	0.0092	—
Grand total	47				

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

smaller male. But why do small defenders call? Most other males are larger than them and high pitched croaks would seem to invite attacks more often than not. Perhaps the reason is that any small defender who remained silent, and was not prepared to give an honest demonstration of his size by croaking, would be immediately under suspicion and would be attacked anyway. We must assume that a small male croaks because it is better than not croaking, partly because it deters those attackers that are smaller than he and perhaps also because the call has other functions such as signalling to the female. Even against large males, small males have a 50% chance of successfully resisting a takeover⁶, so it will always be worth their while to try to maintain amplexus.

We suggest that male anuran calls may often act as a signal of body size, either to other males in agonistic encounters or to females in those species that have well developed mating calls. In mammals, low pitched sounds have also become ritualised as threat signals which may communicate body size or fighting ability and thus permit competing males to assess each other without recourse to costly fights^{15,16}.

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Inverse relation of survival of lizards with island size and avifaunal richness

SMALL islands have fewer species than larger islands or mainlands, and the number of species of very large predators, for example raptorial birds, may be disproportionately lower^{1,2}. This suggests the reasonable but largely untested idea that predation is less important as a determinant of population structure on small islands than on large islands or mainlands, particularly for relatively large potential prey such as songbirds and lizards^{3–10}. We have compared the survival of three species of the lizard *Anolis* on two islands differing in size and number of predatory bird species. We report here that annual survival in sites identical in diurnal lizard species and nearly so in vegetation structure is smaller, and usually substantially so, on the larger island. In one species, the size distribution of males parallels this difference in survival: lizards are smaller where survival is lower. Sexual differences in survival between the two islands are especially marked in an intensely territorial, polygamous species.

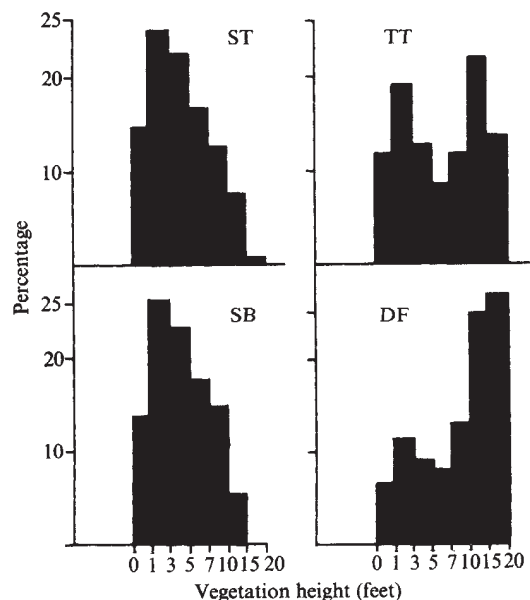
Species of *Anolis* are the most abundant and conspicuous vertebrates on most Caribbean islands. Andros, the largest

island in the Bahamas (4.1×10^3 ha), is in effect a mainland for such lizards, whose home ranges average several m^2 . South Bimini, our second island, is 1,300 ha and has less than half the number of breeding species of predatory birds as Andros (10 compared with 24). It has half the number (seven compared with 14) of large predatory species (≥ 25 cm, about the body size of the mockingbirds, *Mimus*), and some are very rare, for example the mockingbirds^{11,12}. In contrast, the two islands have the same species of diurnal lizards (and snakes), probably because of the smaller spatial requirements of poikilotherms than homeotherms of the same mass^{13–15}. Bimini is the smallest island that is identical to Andros in this way¹⁶.

We selected two sites on each island such that vegetation profiles were almost identical (Fig. 1; compare especially the short-blackland and short-tree sites). Sites were studied during approximately the same period. At the beginning and end of this period, individuals of *A. sagrei*, *A. distichus* and *A. angusticeps* were marked in each site until fewer than 5% were estimated to be unmarked. *A. carolinensis* was also present at all sites but too rare to use in statistical comparisons.

Our results (Fig. 2) strongly support the hypothesis that survival is lower on Andros than on the much smaller Bimini. Because in these species the sexes sometimes differ in rates of survival while within each sex survival is exponential (as we shall show elsewhere), rates of survival are separated by species and sex but not by age. In six out of six cases lizards survived better in the short-blackland site (Bimini) than the short-tree site (Andros) ($P=0.016$). In five out of six cases lizards survived better in the short-blackland site than in the tall-tree site (Andros) ($P=0.109$). Three comparisons (*A. sagrei* males, both sexes combined of *A. distichus* and both sexes combined of *A. angusticeps*) are significant ($P<0.05$, 0.05 and 0.01,

Fig. 1 Foliage profiles for the four study sites. ST, Short-tree (Andros); TT, tall-tree (Andros); SB, short-blackland (Bimini); DF, deep-forest (Bimini). Histograms give surface area of all branches and trunks ≥ 2.54 cm diameter between 0 and 6.1 m. Methods for estimating these distributions were described in ref. 21; in all cases, at least half the surface area of the study site was sampled. Two sites (left column) are virtually identical in profile, but differ somewhat edaphically (the Andros site is rockier than the Bimini site). All vegetation is classified floristically as incipient or developed blackland forest²². Sites were 7.6–9.1 m $\times$ 15.2–18.3 m, about the areas most of whose *Anolis* can be marked in 2 weeks. Andros sites, between Congo Town and Drigg's Hill, were studied between 18 February and 1 March 1973 and between 15 and 25 February 1974. Bimini sites were studied between 15 March and 10 May 1973, and between 29 April and 13 May 1974.



respectively, in one-tailed χ^2 tests). Comparison of somewhat more dissimilar vegetation gives similar results: in six out of six cases, lizards survived better in the deep-forest site (Bimini) than in the tall-tree site ($P=0.016$). For male *A. sagrei*, the comparison is significant ($P<0.01$). The single exception is for female *A. sagrei* in one of the sites on Andros; they survive about as well as female *A. sagrei* on Bimini.

The substantially greater survival of *A. sagrei* females than males on Andros is predictable from the social system of *A. sagrei*. Large males are polygamous and aggressively territorial. Although females are also territorial, they are less conspicuously so. A relatively greater death rate for males in a site with greater risk is therefore not surprising. The survival on Andros of *A. distichus* and *A. angusticeps*, which are less intensely territorial, is consistent with this explanation: on average the

survival of males and females is about equally depressed and is not as low as that of *A. sagrei* males. The greater survival of *A. sagrei* females compared with the more arboreal species may result because their habitat—low understory—is frequented less by birds.

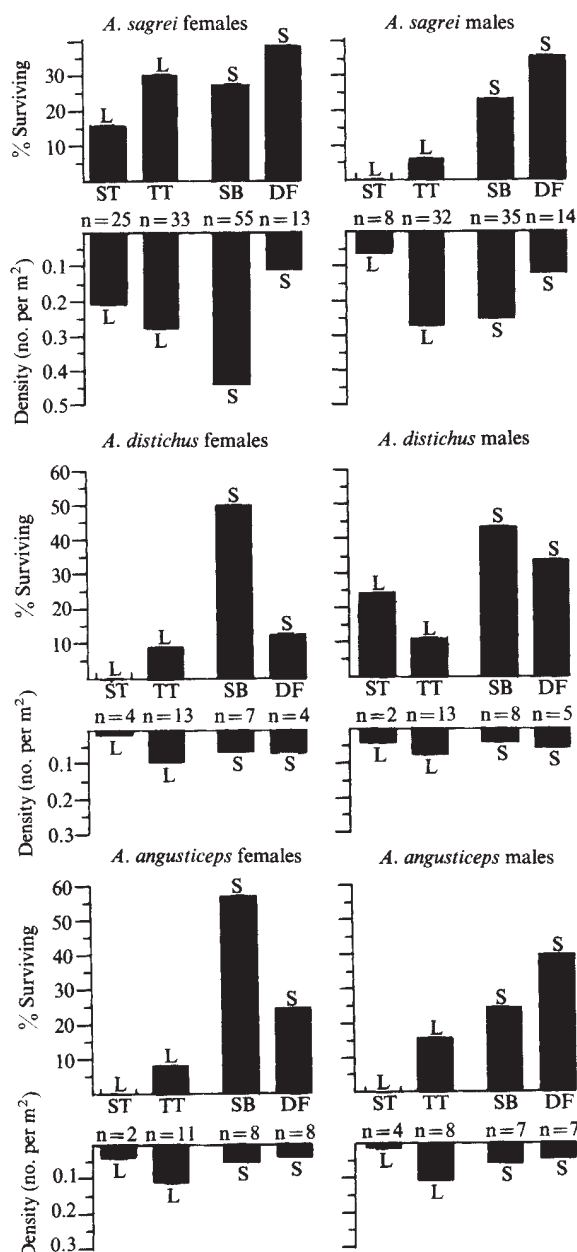
Differences in life expectancies on the two islands should affect body sizes. For the most conservative comparison possible, we used the two sites with the least size difference (tall-tree, years combined, compared with short-blackland, 1973 only). To ensure that differences were not merely due to differences in dates of sampling, we used growth equations (based on Bimini data collected during 3 months¹⁷) to reduce Bimini sizes to values that would have been obtained at the time we were working on Andros. Only male and female *A. sagrei* had significantly greater mean size on Bimini than on Andros ($P<0.02$ and $P<0.05$, respectively). The difference between males (43.5 mm compared with 39.0 mm) exceeded that between females (35.4 mm compared with 33.7 mm). Males also reached a much greater maximum size on Bimini (51 mm compared with 57 mm), and as males can grow larger on Bimini than on Andros in less than 1 yr, the size difference could not result merely from a difference in age. While selection should favour smaller size among individuals with shorter life expectancies, why have not *A. sagrei* females or especially the other species diminished as much in size? Perhaps sexual selection forced the size of male *A. sagrei* on bird-poor Bimini substantially higher than that optimal for activities such as feeding. Female *A. sagrei* and the other species, because of a lesser premium on aggressive success, may be relatively close to the optimum size everywhere. A substantial increase in risk would then differentially favour size reduction in *A. sagrei* males.

Our experimental design rules out greater competition from diurnal lizards as the cause of lower survival on Andros: the same species are present on the two islands and relative abundances are similar. We can also probably rule out predation from snakes, the only important large predators on these islands other than birds, because species lists are identical for the two islands. Nor is differential emigration likely to be the cause of differential survival. By monitoring transient influxes of males and females of our three species in several sites, including one of those discussed here, we have shown that the number of individuals that are itinerant and the distance moved in a year do not correlate with survival. Mortality therefore seems to be more important; indeed, emigration of an established lizard may be a prelude to its death.

Although the greater richness of the larger island's predatory avifauna seems thus the only reasonable cause of differential mortality, it is not certain whether death is caused by predation by birds, competition with them, or both. Compared with Andros, Bimini has both fewer species likely to be able to consume at least a medium-sized (35–40 mm) lizard and fewer smaller species, which are more likely to be deleterious as competitors. Moreover, numbers of both kinds of species are lower in about the same proportion. Predation on these widely spaced lizards is almost unobservable: few can be watched simultaneously, the number disappearing per day is small (0.7% for *A. sagrei* males in the tall-tree site), and large predators are wary. However, a separate analysis of the incidence of broken tails supports a higher intensity of predation on Andros. Moreover, the fact that we saw no obviously emaciated lizards and that males of the most conspicuously aggressive species are especially affected on Andros weakly supports predation as the primary proximate cause of mortality. But perhaps the two processes should not be separated. An immediate effect of a reduction of food by competitors may be that individuals feed longer and thereby increase the risk of being eaten¹⁸; competition may be the ultimate factor.

Because of lower survival lizards might be expected to be sparser everywhere on Andros. But densities of nearly all classes in the most crowded sites (tall-tree and short-blackland) on the two islands are usually fairly similar (Fig. 2). This may

Fig. 2 Survival (top) and density (bottom) in four sites on small (S) and large (L) islands. ST, short-tree (Andros); TT, tall-tree (Andros); SB, short-blackland (Bimini); DF, deep-forest (Bimini). Survival measured from 1973 to 1974. Density is from 1973 only; to compute densities lizards were considered present in an area if the geometric centres²³ of their home ranges were within site boundaries (the same convention was followed for size comparisons in the text).



indicate greater birth rates on Andros, but in view of the probable depletion of food by a more diverse avifauna, a different explanation seems likelier. In the most favourable habitats on any island, the proximate population regulator may be intraspecific territoriality. In such sites, lizards artificially or naturally removed are replaced rapidly by others^{19,20}. On Bimini, where survival is high, lizards occur at relatively high densities in areas apparently unfavourable for feeding and thermoregulation. On Andros, where survival is low, sites favourable in this way act as sinks, draining individuals from less favourable areas. On large islands such as Andros steep density gradients should result, so that areas with almost no lizards are speckled by oases of lizards at high densities. Comparison of densities of the two sites on Andros (Fig. 2), one (short-tree) poorer than the other but vegetationally similar to the short-blackland site on Bimini, gives this idea some support. Female *A. sagrei*, which showed the least survival differential in our study, is, as expected, the only exception.

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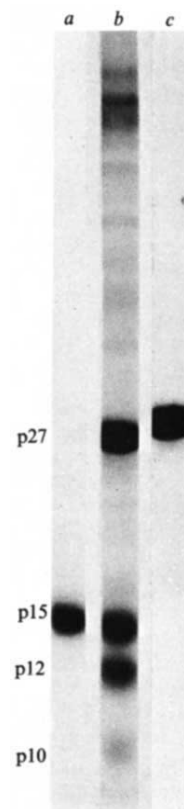
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Abrogation of lymphocyte blastogenesis by a feline leukaemia virus protein

THYMIC atrophy and suppression of cell-mediated immunity (CMI) have been associated with feline leukaemia virus (FeLV) infection in cats¹⁻⁵. Impairment of CMI by FeLV and other oncogenic retroviruses has been attributed to impaired lymphoid function caused by viral infection of lymphoid tissues^{5,6}. This explanation for FeLV-related immunosuppression is plausible as lymphoid cells are the targets for oncogenesis^{3,7} and atrophy of the thymus occurs early in the course of FeLV infection^{1,3,4}. However, FeLV-associated immunological impairment is not due simply to viral destruction of the thymus as surgical thymectomy of kittens does not result in demonstrable immunosuppression⁸. Moreover, several observations indicate that the depressed CMI response of preleukaemic FeLV-infected cats may be due to T-cell unresponsiveness rather than T-cell deficiency: the circulating T-cell population is not decreased during the preleukaemic stage of the FeLV infection²; lymphocytes from FeLV-infected

Fig. 1 SDS-polyacrylamide gel electrophoresis of FeLV and purified p15 and p27. Gels consisted of 7.5% acrylamide with 5% bisacrylamide in a Tris-acetate buffer containing 0.205 M Tris, 0.205 M acetic acid and 0.1% SDS, pH 6.6. Protein samples were mixed with equal volumes of 0.02 M Tris, 0.02 M acetic acid, 0.1% SDS and 0.1 M dithiothreitol, and heated to 100°C for 3 min before electrophoresis. Gels were electrophoresed for 5 h at 8 mA per column using constant current. *a*, Purified p15 from FeLV; *b*, whole FeLV; *c*, purified p27 from FeLV. Production and purification of R-FeLV has been described⁹. The only addition to the procedure was that purified virus was banded a second time on linear sucrose gradient. To purify p15, FeLV ($\geq 10^{11}$ particles ml⁻¹) was twice frozen-thawed and centrifuged at 100,000g for 90 min using an SW27 rotor in a Beckman L2 65-B ultracentrifuge. Pellets were resuspended in 2 ml TKE-D-Tx buffer (0.05 M Tris-HCl, 0.6 M KCl, 0.01 M EDTA, 0.01 M dithiothreitol, 1% Triton X-100) pH 7.2, incubated at 37°C for 1 h and centrifuged at 100,000g for 90 min. The liquid phase was collected and extracted 8-10 times with 10-15 volumes of ether to remove the Triton X-100. Following the final extraction, residual ether was removed by blowing a fine stream of nitrogen across the surface of the liquid. The remaining solution was again centrifuged at 100,000g for 10 min. The liquid phase (fraction *a*) was collected for later use and the pellet (fraction *b*) redissolved in TKE-D-Tx buffer. p15 was purified from fraction *b* by liquid column chromatography using a 2.5×90 cm Sephacryl 200 (Pharmacia) column equilibrated with modified TKE-D-Tx buffer (0.01 M Tris HCl, 0.3 M KCl, 0.01 M EDTA, 0.01 M DTT, 1% Triton X-100), pH 7.2. A single major peak of highly purified p15 was resolved by this procedure. p27 was purified from fraction *a* by liquid column chromatography using a 2.5×90 cm Sephadex G150 column equilibrated with TKE-D buffer (0.01 M Tris, 0.3 M KCl, 0.01 M EDTA, 0.01 M DTT), pH 7.2.



cats are unresponsive to phytohemagglutinin-induced lymphocyte blastogenesis (LBT)²; and inhibition of the LBT response of normal feline lymphocytes can be produced by serum from FeLV-infected cats⁹. We have found that the unresponsiveness of feline lymphocytes to concanavalin A (Con A) also can be induced with FeLV inactivated by ultraviolet light¹⁰. Similar depressed mitogenic responses have been reported for mouse lymphocytes incubated with disrupted murine leukaemia virus¹¹. Here we report that the mediator of FeLV-associated lymphocyte unresponsiveness seems to be the 15,000 molecular weight virion protein (p15) of FeLV.

Table 1 Inhibition of Con A-LBT by p15 and p27

Cat	Inhibitory protein	% Inhibition*
550	p15	51.8±8.8†
555	p15	45.1±6.6
281	p15	92.0±17.9
679	p15	92.9±16.3
795	p15	61.5±7.9
760	p15	67.3±11.4
550	p27	15.2±8.7
555	p27	20.8±10.3

Cat lymphocytes (1×10^5 cells) from SPF cats were incubated with 5 µg p15 or p27 and 10 µg Con A in quadruplicate tests for 5 d. During final 18 h of incubation, 0.5 µCi ³H-thymidine was added.

* Mean c.p.m. of cells incubated with Con A plus inhibitory protein (p15 or p27) mean c.p.m. of cell incubated with Con A alone.

† S.d. of the mean expressed as percentage.

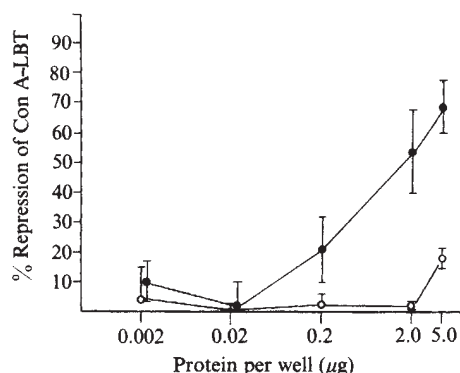


Fig. 2 Tests for inhibition of Con A-induced LBT of cat lymphocytes incubated with p15 or p27. Dilutions of p27 or p15 were incubated with Con A (10 µg) and 1×10^5 peripheral blood leukocytes (PBL) (in quadruplicate) for 5 d. Points represent the mean value of tests run on six cats for p15 and two cats for p27. Vertical lines represent s.e.m. ●, Test incubated with p15; ○, test incubated with p27. PBL were isolated by centrifugation of heparinised blood taken from 6–12-month-old normal SPF cats through Ficoll-Hypaque gradients. The LBT assay was a modification of that described by Cockerell *et al.*²⁰. Briefly, 0.1 ml PBL cell suspensions (1×10^6 lymphocytes) were mixed in Microtest plates (Falcon) with 0.05 ml of an optimal concentration of Con A (10 µg per well) (Sigma) and 0.05 ml of the protein being assayed for LBT inhibition. The plates were incubated at 37 °C for 5 d. The protein preparations being assayed for LBT inhibition had been previously dialysed against three changes of 400 volumes MEM-s medium containing 1% antibiotics. In the Con A control wells, the inhibitory protein was replaced with 0.05 ml complete medium. In cell control wells, both Con A and inhibitory proteins were replaced with complete medium. During the final 18 h of incubation, 0.1 ml of medium containing 0.5 µCi ^3H -thymidine (6.7 Ci mmol^{-1} ; New England Nuclear) was added. Cells were collected on glass filter paper with a semiautomatic multiple processor (Otto Heller) and assayed for radioactivity using a liquid scintillation counter. Net c.p.m. of quadruplicate wells were averaged to obtain mean c.p.m.

Purified p15 and p27 (the 27,000 structural proteins of FeLV) were tested for inhibitory effect on the Con A-LBT response using lymphocytes from normal specific pathogen-free (SPF) cats (Fig. 1). When purified p15 was incubated with Con A-stimulated feline lymphocytes from six cats, the magnitude of blast transformation, as determined using ^3H -thymidine uptake, was reduced by 45–92% of that obtained with control cultures free of p15 (Table 1). When p27 was substituted for p15 in parallel tests, however, no significant inhibition was detected. LBT inhibition data for various concentrations of p15 and p27 are given in Fig. 2. p15 was inhibitory at a concentration of 0.2 µg per well (or more).

Depression of ^3H -thymidine uptake in the LBT assay could be caused either by lymphocyte unresponsiveness, by toxicity, or by competitive inhibition of Con A binding. To determine

Fig. 3 Inhibition tests using lymphocytes from two cats in which 5 µg per well of p15 was added at various times during the LBT assay. Each point is the mean of quadruplicate tests. The vertical line represents the s.e.m.

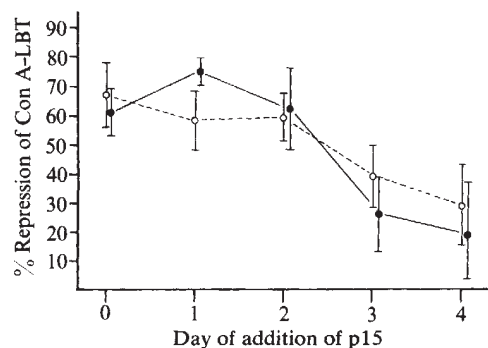


Table 2 Effect of increased concentration of Con A on p15-induced LBT inhibition

Cat. no.	% Inhibition of LBT at Con A concentrations* of:		
	10	25	50
175	98.2 ± 0.1†	95.3 ± 1.3	92.5 ± 0.7
288	99.5 ± 0.1	99.0 ± 0.2	95.2 ± 0.1
28	98.7 ± 0.4	98.6 ± 0.2	97.5 ± 0.3
77	99.7 ± 0.1	99.6 ± 0.1	99.5 ± 0.1

Lymphocyte cultures were set up for LBT using optimal (10 µg per well) and increasing concentrations of Con A (25 and 50 µg per well); 5 µg p15 was added to each well.

* µg per well.

† Percentage inhibition of Con A-LBT ± s.e.m.

whether p15 is toxic to feline lymphocytes, lymphocytes from two normal SPF cats were incubated for 5 d with a p15 concentration of 0–5 µg per well. Lymphocyte viability was essentially the same for all concentrations of p15. To determine whether p15, in conjunction with Con A, is toxic to dividing lymphocytes, p15 was added at various periods following the addition of Con A (Fig. 3). When p15 was added on day 0, 1 or 2, approximately the same level of inhibition was attained. Addition of p15 on day 3 produced less inhibition and addition on day 4 did not produce inhibition of the LBT response. These results suggested that p15 was not toxic to Con A-stimulated lymphocytes because addition of p15 on day 4 did not reduce the level of ^3H -thymidine uptake. To determine whether p15 is a competitive inhibitor of Con A binding, the concentration of Con A in LBT assays was increased by factors of 2.5 and 5. Increasing the concentration of Con A, however, had no effect on the level of p15-mediated LBT inhibition (Table 2).

The temporal requirement for optimal p15 inhibition of Con A-LBT seemed to be within the first 48 h of Con A stimulation (Fig. 3). During this period, a number of Con A-induced cellular functions occur, including Con A binding, alterations in cytoplasmic membranes, and DNA and RNA synthesis. It is not yet possible to identify the exact cellular functions affected by p15.

To extrapolate from this unresponsive state as measured by *in vitro* assays for lymphocyte-mitogen interactions to true *in vivo* immunosuppression associated with FeLV infections is tenuous although similar correlations have proven valid in other systems¹². It is clear, however, that immunosuppression usually is found in cats with FeLV-induced malignancies and that abrogated immune functions could account for the progressive nature of the disease.

Studies with MuLV disclosed a similar virion protein which depresses lymphocyte function (A. Hellman and A. Fowler, personal communication). Others have found substances in tumour homogenates that interfere with *in vitro* assays for cellular immunity^{13,14} and in two recent *in vivo* studies, inoculation of attenuated MuSV¹⁵ or ultraviolet light-inactivated FeLV^{16,17} into mice and cats, respectively, appeared to abrogate normal immune function.

The concept that virion structural proteins per se can interfere with normal cell functions has precedence. The adenovirus 12 penton fibre protein suppresses DNA synthesis when incubated with cell culture¹⁸. It is possible that FeLV, and perhaps other retroviruses, carry a structural component which interferes with local immunological functions at the site of tumorigenesis and eventually may mediate generalised immunosuppression.

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Fusion of Sendai virus with the target cell membrane is required for T cell cytotoxicity

INFECTION of mice with viruses can generate cytotoxic T lymphocytes (CTL) which show restricted specificity for target cell lysis. Specific lysis requires that the virus used to prime the target cells must be of the same type as that used to sensitise the CTL, and that both target and CTL cells must express the same major histocompatibility complex (MHC) gene product(s). The nature of the viral gene product(s) and their interaction with the MHC gene product(s) have been the subject of recent study¹⁻⁵. Previously we used Sendai virus to show that lysable target cells can be obtained using membrane vesicles which contain only the viral glycoproteins, indicating that these may be the specific viral gene products involved in target formation⁷. Sendai virus contains two glycoproteins—the haemagglutinin-neuraminidase (HANA) which promotes attachment of virus to cells and the fusion protein (F) which is involved in subsequent virus cell fusion⁷⁻⁹. Both activities are necessary for insertion of these viral glycoproteins into the plasma membrane of the cell¹⁰. In this letter we suggest that the insertion of the viral glycoproteins into the cell membrane is an essential step in target cell formation since we can show that virus containing an inactive fusion protein precursor (F₀) cannot elicit T cell cytotoxicity unless the fusion activity is generated by proteolytic cleavage of the precursor. Sugamura *et al.*⁶ have suggested that it is primarily the F glycoprotein of the Sendai virus envelope which is essential for the formation of the target antigen, as virus lacking the functional activities of F following trypsin digestion was inactive in priming target cells for T cell killing. However, we show that proteolytic inactivation of either of the two glycoproteins (F or HANA) of virus used to prime target cells will abolish the cytotoxic response.

Virus-specific effector cells bearing the surface markers Thy 1⁺, Ly 2⁺2⁺ and L 6⁺ (M. Horton *et al.*, in preparation) were generated *in vitro* by secondary stimulation⁵ of *in vivo* primed mouse spleen cells with noninfectious (β -pro-

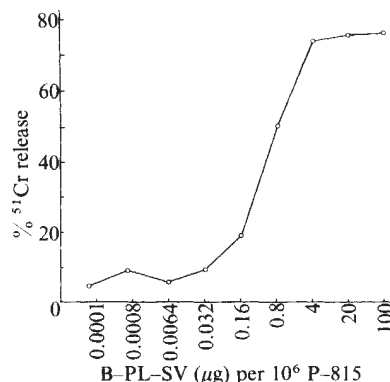


Fig. 1 Dilution curve for Sendai virus-specific T cell-mediated cytotoxicity. P-815 cells were incubated with β -propiolactone-treated Sendai virus for 1 h at 37 °C and washed three times with medium. Susceptibility to Sendai virus specific CTLs was measured by the ⁵¹Cr release assay described previously using 10⁴ target cells per well⁵. Results are from triplicate assays; s.d. were always less than 3. Spontaneous lysis was 12% and has been subtracted. ○, Lysis of ⁵¹Cr labelled P-815-Sendai virus treated cells; ●, lysis of untreated P-815 cells.

piolactone-inactivated) Sendai virus (20 μg virus protein per 10⁶ cells). In experiments designed to calibrate the cytotoxic assay, the degree of lysis was shown (Fig. 1a) to be related to virus concentration over the range 0.16–4 μg of virus protein using 10⁶ P-815 target cells, corresponding to approximately 10⁴–10⁵ virus particles per cell. Amounts of virus greater than 4 μg did not increase the degree of lysis. Thus investigations of changes in target formation by different virus preparations must be carried out in a concentration range which gives a linear response in the assay.

In the experiment described above and in previous work⁵ we have used egg-grown Sendai virus which is active in fusion. To investigate further the role of virus-cell fusion in target cell formation we purified Sendai virus produced in MDBK cells. This virus contains F₀ and is inactive in fusion and non-infectious although it contains an active HANA glycoprotein and can attach to the cell surface⁹. Activation of fusion requires proteolytic cleavage of the F₀. When Sendai virus is grown in embryonated eggs this cleavage presumably occurs at the plasma membrane. In MDBK cells this cleavage does not occur but MDBK grown virus can be cleaved *in vitro* by low concentrations of trypsin with restoration of the fusion and haemolytic activities and also of infectivity⁹. In Table 1 the haemagglutinin, neuraminidase, haemolytic and target formation activities of trypsin treated MDBK-Sendai are compared with those of untreated MDBK-Sendai and egg-grown Sendai virus. MDBK-Sendai does not cause significant cell-mediated cytotoxicity (CMC) unless it is activated by pretreatment with trypsin. The small amount of target formation obtained with MDBK-Sendai is probably due to the small proportion of cleaved F which is always found in these virus preparations. These results suggest that MDBK-Sendai virus will not form lysable target cells because the virus and hence the viral glycoproteins cannot fuse with the cell membrane. To show that the glycoproteins of virus containing F₀ can function in target formation if they are present in the cell membrane, P-815 cells were infected with a low dose (10 EID per cell) of live egg-grown Sendai virus. This input of virus is too low to cause immediate target cell formation but after incubation for 24 h at a time when virus containing F₀ and HANA was being released from the P-815 cells (results not shown) the target cells were specifically lysable. However, P-815 cells treated with P-815 grown Sendai virus were not lysed by T-killer cells unless the virus was activated by pretreatment with trypsin to convert F₀ to F (Table 1). These results further support the hypothesis that the process of fusion and membrane insertion of viral glyco-

proteins is a major requirement for target formation, as virus containing F₀ can form targets if the viral glycoproteins are inserted in the cell membrane from within the cell.

Although these results suggest that T cell-mediated cytolysis requires the presence of viral antigens in the plasma membrane it is possible that the T cells might recognise viral antigens that are bound to but not fused into the cell surface. In order to try to distinguish a recognition phase from a killing phase we have used unlabelled target cells having viral antigens either fused into the membrane or merely attached to the cell surface to compete with ⁵¹Cr labelled Sendai-treated target cells for lysis by T killer cells. The results shown in Fig. 2 indicate that cells coated with MDBK-Sendai virus do not compete for lysis of labelled targets to a greater extent than do normal cells at the same density. In contrast, target cells treated with trypsinised MDBK-Sendai virus or with egg-grown Sendai virus will compete effectively. Thus attachment of virus to target cells is insufficient for T-cell recognition as well as for CMC.

To investigate further the involvement of the Sendai virus glycoproteins in target formation we used limited proteolytic digestion of β -propiolactone-treated virus to selectively inactivate the F or HANA glycoproteins. The effect of the protease treatments on the viral activities is shown in Table 2. Digestion of virus with pronase or the V8 protease of *Staphylococcus aureus* eliminates the haemagglutinin and neuraminidase activities, while treatment with trypsin (a concentration higher than that required for precursor cleavage) causes only a small decrease in these activities. All enzymes abolish the fusion and haemolysis activities of the virus. Loss of HA activity prevents attachment of virus to cells and thus prevents the subsequent virus-cell fusion even if the fusion protein is active. Using polyacrylamide gel analysis we have shown that pronase cleaves both F and HANA while trypsin cleaves F and the V8 protease removes the HANA. The fusion protein of virus treated with V8 protease is indistinguishable from that of untreated virus by analysis on polyacrylamide gels, immunological analysis and amino acid composition (M.-J. G. and M. W., in preparation). Target formation by the protease treated virus preparations was evaluated after incubation of 10⁶ P-815 cells with modified or untreated virus. The results shown in Table 2 indicate that inactivation of not only F but also HANA abolishes the capacity of virus to form target cells or reduces it by more than 99%.

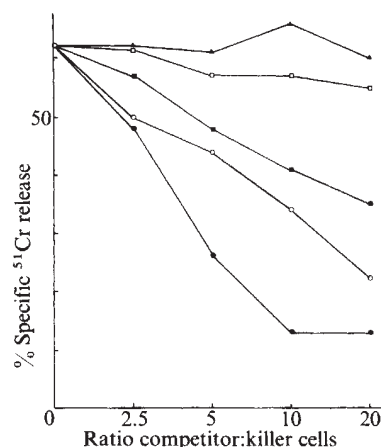


Fig. 2 Inhibition of T cell-mediated cytolysis of Sendai virus-treated ⁵¹Cr-labelled P-815 cells by competitor P-815 cells treated with various Sendai virus preparations. Competitor cells: ▲, P-815 control; □, P-815+MDBK-Sendai virus (100 μ g per 10⁶ cells); ■, P-815+trypsin treated MDBK-Sendai virus (100 μ g/10⁶ cells); ○, P-815+egg Sendai (10 μ g per 10⁶ cells); ●, P-815+egg Sendai (100 μ g per 10⁶ cells). The cytotoxic test was carried out as described in Fig. 1. Killer: target cell ratio 10:1.

The results presented here show that the capacity of Sendai virus to fuse with a target cell is essential for T-cell cytotoxicity. This process of fusion results in both the insertion of the viral glycoproteins into the cell membrane and also of the viral capsid into the cell. We previously reported that noninfectious Sendai virus (inactivated by UV light or β -propiolactone treatment) or artificial vesicles containing only the two glycoproteins were efficient in target cell formation⁵. We therefore suggest that the glycoproteins must be inserted into the cell membrane for target formation. We also show that inactivation of either of the two glycoproteins of Sendai virus will prevent target formation and this interference can be explained by a block at the level of virus-cell fusion. These results extend the observations of Sugamura *et al.*⁶. Such experiments cannot

Table 1 Effect of trypsin treatment on the glycoprotein activities of MDBK-Sendai virus

Virus sample	Cytotoxic assay			Haemolysis activity (A ₅₄₀ per 20 μg)	Haemagglutinin activity (HAU per 20 μg)	Neuraminidase activity (A ₅₄₉ per 20 μg)
	μg virus	% ⁵¹ Cr release killer: target ratio				
		20:1	5:1			
Expt 1						
Egg-grown Sendai virus	1	82	74	1.44	2,000	1.18
	10	90	78			
MDBK-grown Sendai virus	10	5	2	0.07	2,000	1.08
	100	15	14			
Trypsin treated MDBK-Sendai virus	100	80	70	1.19	2,000	1.04
Expt 2						
Egg-grown Sendai virus	1	23	18	1.01	2,000	0.99
	10	42	33			
	100	49	35			
P-815 grown Sendai virus	10	0	1	0.02	2,000	1.04
	100	3	2			
Trypsin treated P-815-Sendai virus	10	8	5	0.45	2,000	0.95
	100	15	4			

Purified β -propiolactone treated MDBK-Sendai virus or P-815-Sendai virus (1 mg in 2 ml phosphate-buffered saline (PBS), pH 7.2) was incubated with trypsin (2.5 μ l, 5 mg ml⁻¹ in 1 mM HCl) for 10 min at 37 °C. The reaction was terminated by addition of soybean trypsin inhibitor (10 μ l, 10 mg ml⁻¹ in PBS). Control samples of MDBK-Sendai virus and P-815-Sendai virus were treated in exactly the same manner except for the addition of trypsin. Haemagglutinin, neuraminidase and haemolytic activities were assayed as described previously⁵. The cytotoxic test was carried out as described in Fig. 1 legend. Spontaneous lysis from targets was 9–13%. Release from noninfected targets was subtracted. The addition of soybean trypsin inhibitor had no effect on the cytotoxic assay. The cytotoxic assays in Expt 2 were carried out with a different batch of CTLs which were less active in cytolysis.

Table 2 Effect of proteolytic digestion on activities of Sendai virus

Protease treatment	μg virus	Cytotoxic assay		Haemagglutinin activity (HAU per 20 μg)	Neuraminidase activity (A_{549} per 20 μg)	Fusion activity (per 20 μg)		Haemolysis activity (A_{540} per 20 μg)
		% ^{51}Cr release	killer: target ratio			Average no. nuclei per cell	% of nuclei in poly-karyocytes	
		20:1	5:1					
a, No enzyme	0	7	5					
	1	86	58	2,048	1.01	5.2	94	1.86
	10	90	62					
b, Trypsin	10	10	7					
	100	7	2	2,048	0.94	0	0	0.02
c, V8 protease	10	10	6					
	100	30	12	32	0.03	0	0	0.01
d, Pronase	10	8	8					
	100	14	10	4	0	0	0	0

β -PL Sendai virus (2 mg in 1 ml of the appropriate buffer) was digested with proteolytic enzymes as follows: a, no enzyme; b trypsin (8 μl , 5 mg ml^{-1} ; TPCK-treated, Worthington) for 1 h at 37 °C in 0.5% ammonium bicarbonate buffer, pH 8. c, V8 protease from *Staphylococcus aureus* (50 μl , 1 mg ml^{-1} ; Miles) for 18 h at 37 °C in 50 mM ammonium bicarbonate buffer, pH 8; d, pronase (8 μl , 5 mg ml^{-1} ; Calbiochem) for 4 h at 37 °C in PBS buffer. The capacity of the virus preparations to induce target cell formation was measured in the cytotoxic assay described in Fig. 1. Haemagglutinin, neuraminidase and haemolytic activities were measured as before⁵. For cell fusion measurements replicate newly confluent cultures of Hep 2 cells in 30 mM plastic Petri dishes were inoculated with 0.1 ml of the virus preparation in PBS containing Mg^{2+} and Ca^{2+} , or were mock infected. Following virus adsorption for 1 h at 37 °C the cultures were overlaid with 2.0 ml of Dulbecco's reinforced Eagles medium containing 10% fetal calf serum. After incubation for 2 h at 37 °C in an atmosphere not enriched with CO_2 the number of nuclei per 100 cells were counted for several fields on each dish.

determine which glycoprotein is primarily responsible for target formation, because both HA and F activities are required for virus-cell fusion. Further studies using a temperature-sensitive HANA⁻ mutant of Sendai virus are in progress to elucidate this question.

Insertion of the viral glycoprotein(s) into the cell membrane may be required to facilitate interaction with the MHC gene products. However, at this stage there is no evidence to discriminate between the hypotheses of one or two T-cell receptor mechanisms, that is, whether the viral glycoprotein(s) interact directly with the MHC antigens or whether they act as independent antigens for T-cell recognition.

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Mapping of *H-2* genes associated with T cell-mediated cytotoxic responses to SV40-tumour-associated specific antigens

T CELL-MEDIATED cytotoxic responses to cells carrying viral antigens¹, haptens² and male H-Y antigen³, as well as minor histocompatibility antigens⁴ are known to be associated with either the *K* or *D* region of the murine major histocompatibility complex (*H-2*). Similarly, T cell-mediated cytotoxicity of SV40-transformed cells expressing tumour-associated specific antigens (TASA) is restricted to syngeneic target cells⁵⁻⁸. Although good cytotoxic responses to SV40-transformed cells can be found in mice of *H-2^b* haplotype after primary *in vivo* immunisation⁵, the response of mice of other *H-2* haplotypes is quite poor (unpublished observations), but can be readily demonstrated after *in vitro*⁶⁻⁸ mixed cell cultures. We demonstrate here primary cytotoxic responses to SV40-TASA in various inbred mouse strains using an immunisation protocol previously applied to the primary *in vivo* induction of hapten-specific⁹ and virus-specific cytotoxic T lymphocytes (CTL)¹⁰. Moreover, the availability of appropriate congenic recombinant mice as responders and SV40-transformed cells from these mouse strains as target cells, has enabled us to map the anti-SV40-TASA response within the *H-2* complex. The data establish that the response is restricted to *H-2K* or *H-2D*, and indicate the existence of a possible *Ir* gene influence on the T-cell response to SV40-TASA associated with *H-2D^d*.

B10 and BALB/c mice were immunised with SV40 (legend to Fig. 1). The kinetics of the CTL generation were studied by varying the time of *in vivo* immunisation (Fig. 1) as well as the *in vitro* culture period (Fig. 2). Antigenic specificity of the CTL was investigated using SV40- and adenovirus 5-transformed cells as targets for the ^{51}Cr release assay. Maximum cytotoxic activity was obtained in B10 and BALB/c mice 7 d after *in vivo* immunisation followed by a further 3 d *in vitro* culture period (Figs 1, 2). In accordance with earlier results⁵, CTL could be demonstrated in B10 mice when tested directly 7 d after immunisation, whereas at that time, BALB/c lymphocytes did not mediate specific lysis of the same SV40-transformed (BALB/c $\times$ C57BL/6) F_1 target cells (Fig. 2, day 0 *in vitro*). However, after a 3 d *in vitro* incubation without addition of

antigen, BALB/c lymph node cells developed cytotoxic capacity equivalent to that found for the B10 effectors. The efficiency of B10 CTL was also greatly increased by this procedure (Fig. 2). Target cell lysis was SV40-TASA-specific and syngeneically restricted, as neither adenovirus 5-transformed target cells (Fig. 2) nor allogeneic SV40-transformed cells (Fig. 1) were lysed. Furthermore, cytotoxic activity was abrogated after treatment of immune lymph node cells with anti-Thy 1.2 serum and complement, establishing that the effectors are T cells (data not shown). In additional similar experiments SV40-TASA-specific CTL could also be generated in different mouse strains carrying the $H-2^k$ and $H-2^d$ haplotype (data not given).

It has been shown in some experimental systems that the *in vivo* state of low responsiveness to cell-bound antigens is not due to lack of antigen-reactive T cells, but rather reflects the activity of suppressor T cells which block the *in vivo* differentiation of CTL precursors into CTL¹¹. However, it was noted that mere transfer of *in vivo*-primed lymphocytes to *in vitro* culture conditions seemed to facilitate the development of CTL without the necessity of further antigenic stimulation⁹⁻¹¹. Thus, combined *in vivo* sensitisation/*in vitro* differentiation procedures allow the generation of CTL probably by overcoming suppressor T cell function. Previously reported data suggest that suppressor mechanisms may account for the eclipse in immunity in mice bearing syngeneic SV40-induced tumours^{12,13}. Our results support this interpretation but direct proof awaits further experimental verification.

The experiments described allowed us to investigate possible associations between various allelic forms of $H-2$ -coded molecules and SV40-TASA in cytotoxic responses. The congenic mouse strains B10.BR, B10.D2 and B10 as well as the recombinant strains B10.A, B10.A(2R), (4R), (5R), B10.HTG and C3H.OH were immunised with SV40, and lymph node cells were tested for cytotoxic activity against SV40-transformed target cells at the peak of the response, as described in Figs 1 and 2. Table 1 illustrates that cytotoxic responses to SV40-TASA in B10 mice as well as in B10.A(2R) and (4R) were associated with both the K -region and the D -region of $H-2$. B10 CTL mediated lysis of both B10.A

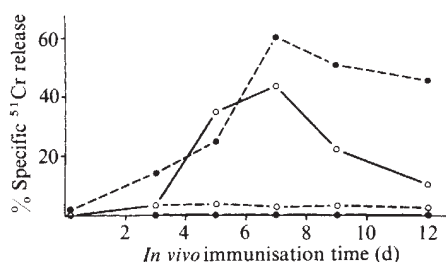


Fig. 1 Time course of *in vivo* sensitisation of SV40-TASA-specific CTL. Groups of three mice from C57BL/10 SgSn and BALB/c were injected at different times with 10^7 plaque-forming units of SV40 into the hind footpads. The draining lymph nodes (LN) from all groups were removed on the same day and cell suspensions prepared in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated fetal calf serum (FCS), 2×10^{-5} M 2-mercaptoethanol and penicillin/streptomycin. 3×10^6 cells in 2 ml supplemented DMEM were plated into each well of Linbro FB16-24TC tissue culture plates and incubated for 3 d at 37°C in humidified air containing 5% CO₂. Thereafter, graded numbers of LN cells (in 100 µl) were tested for cytotoxic activity against 2×10^4 SV40-transformed target cells suspended in 100 µl. The ⁵¹Cr release assay was carried out in triplicate for 5–6 h at 37°C as described⁵. % Specific lysis and standard deviation (s.d.) were calculated as described⁵. Data are given without s.d. as it was usually less than 2%. LN cells from medium-injected control mice did not lyse any of the target cells. The spontaneous ⁵¹Cr release ranged over 10–25%. Data given were obtained at an effector-to-target (E/T) cell ratio of 50:1. ●, LB10SV (SV40-transformed B10 liver cells); ○, KB/c SV (SV40-transformed BALB/c kidney cells); ---, B10 LN effector cells; —, BALB/c LN effector cells.

(5R)SV and (2R)SV target cells, indicating response at K^b and D^b , respectively, whereas B10.A(2R) and (4R) effector cells lysed SV40-transformed target cells with K^k as well as with D^b association (for example, B10.BRSV, C57SV, HTGSV). In contrast, B10.D2 mice failed to respond at K^d , and were found to operate only in the context of D^d . Similarly, B10.HTG mice, which differ from B10.D2 only in the D -region, did not respond in association with K^d , but showed a normal response at D^b . The reciprocal result was found in B10.BR mice. Here, a strong cytotoxic response is associated with the K^k -region, whereas no response could be obtained at D^k (C3H.OH target cells were not lysed).

Thus, it seems that the cytotoxic response to SV40-TASA is associated with K^b , K^k , D^b and D^d but not with K^d and D^k . This nonresponsiveness to SV40-TASA at K^d and D^k was confirmed by testing SV40-injected C3H.OH mice for cytotoxic activity. Cells from these mice did not lyse B10.D2, C3H or C3H.OH SV40-transformed target cells (Table 1), although all targets express the relevant tumour-specific antigens and alloantigens (data not shown). Similarly, lack of cytotoxic responses in association with certain $H-2K$ or $H-2D$ structures has been described for murine tumour viruses¹⁴, for the H-Y antigen¹⁵, and for conventional infectious viruses¹⁶. So far, the reasons for the observed nonresponsiveness are largely unknown. Whether this phenomenon is due to a lack of the T cell clones expressing the relevant receptor(s) or whether it represents a preferential association of SV40-TASA with only certain $H-2$ structures, as has been suggested in other experimental models^{15,17}, remains unclear.

Table 1 Mapping of $H-2$ genes associated with the cytotoxic response to SV40-TASA in different inbred mouse strains

Effector cells*	Mouse strain	SV40-transformed target cells† $H-2$ type		% Specific lysis‡	Mapping of SV40-TASA association
		K	D		
B10	C57BL/6J	b	b	36	K^b, D^b
	B10.D2	d	d	—2	
	B10.A(2R)	k	b	20	
	B10.A(5R)	b	d	22	
B10.A(2R)	B10.A(2R)	k	b	46	K^k, D^b
	B10.BR	k	k	53	
	B10.HTG	d	b	17	
	B10.A(5R)	b	d	0	
B10.A(4R)	B10.A(4R)	k	b	60	K^k, D^b
	B10.BR	k	k	60	
	B10.HTG	d	b	25	
	B10.A(5R)	b	d	0	
B10.D2	B10.D2	d	d	27	D^d
	C57BL/6J	b	b	4	
	B10.A(5R)	b	d	21	
	B10.HTG	d	b	3	
B10.BR	C3H/HeJ	k	k	30	K^k
	B10.D2	d	d	0	
	B10.A(2R)	k	b	66	
	C3H.OH	d	k	5	
B10.HTG	B10.HTG	d	b	28	D^b
	B10.D2	d	d	5	
	C57BL/6J	b	b	44	
	B10.A(2R)	k	b	64	
C3H.OH	C3H.OH	d	k	0	—
	C3H.OH	d	k	1	
	B10.D2	d	d	—4	
	C3H/HeJ	k	k	6	

* Groups of three mice were immunised with SV40 and graded numbers of LN cells were assayed against ⁵¹Cr-labelled SV40-transformed target cells as described in Fig. 1. Data given were obtained at an E/T ratio of 20:1.

† Target cells from the strains indicated were obtained by transformation of kidney or liver cells with SV40 virus.

‡ Spontaneous ⁵¹Cr release was less than 22%.

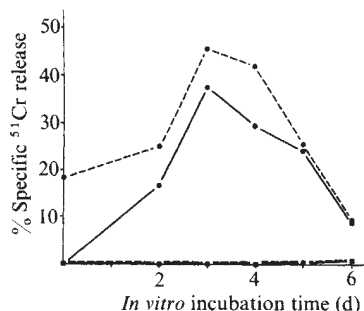


Fig. 2 Time course of *in vitro* differentiation of SV40-TASA-specific CTL. Groups of three mice of C57BL/10 SgSn (B10) and BALB/c were primed *in vivo* with SV40 for 7 d as described in Fig. 1 and LN cells cultured *in vitro* for various lengths of time. To exclude the possibility of nonspecific activation of LN cells during this incubation period due to FCS, cultures were set up in DMEM supplemented with 0.5% autologous mouse serum. The experiment was timed so that all cultures were terminated on the same day and LN cells tested for cytotoxic activity against target cells from (C57BL/6J × BALB/c)F₁ mice either transformed with SV40 (KF₁SV, ●) or adeno 5 virus (KF₁A, ■). The spontaneous lysis of target cells during the 4-h ⁵¹Cr release assay was less than 20%. ----, B10 effector cells; —, BALB/c effector cells.

Responsiveness to SV40-TASA in B10.D2 mice was shown to be associated with *D^d* (Table 1). However, B10.A and B10.A(5R) mice did not generate CTL specific for SV40-TASA cells expressing *D^d*, though they respond well to SV40-transformed target cells at *K^k* and *K^b*, respectively (Table 2). This failure to respond at *D^d* was not due to improper display of antigens on the target cell, as both SV40-transformed B10.D2 and B10.A(5R) targets were lysed by B10.D2 anti-SV40-TASA effector cells (Table 2). Thus, there seem to be additional gene(s) within the *H-2* complex regulating cytotoxic responses to cells expressing both *D^d* and SV40-TASA. As the three strains tested have identical haplotypes from *I-C* to the right as well as identical non-*H-2* background genes, the gene(s) responsible for generation of SV40-specific CTL associated with *D^d* must be located to the left of *I-C*. Although there are no recombinant mice available that would allow the mapping of the exact region involved, one might assume that the differential responsiveness of B10.D2 compared with B10.A and B10.A(5R) mice to SV40-TASA on cells expressing *D^d* is an effect of *I*-region-associated *I*r gene(s) as described for cytotoxic responses to trinitrophenyl-modified cells¹⁸, to the male H-Y antigen¹⁵ and infectious viruses¹⁶.

The mechanism by which *I*r genes may regulate cytotoxic responses is still unclear. However, there is increasing evidence that T cell help is crucial for the generation of CTL^{19,20} and moreover, these T-T cell interactions seem to be *I*-region-

restricted²⁰. Thus, if T cell help is necessary in SV40-TASA responses, and *I*-region genes determine the ability of helper cells to interact with the relevant T cell clones, then B10.D2, B10.A and B10.A(5R) mice should each have helper cell populations which can specifically interact only with precursors of CTL carrying receptors for *D^d*, *K^k*, and *K^b*, respectively, and not with others. Interestingly, the *I-A* and *I-B* regions of B10.D2, B10.A and B10.A(5R) are each of the same haplotype as the respective SV40-TASA-associated *K* or *D* region (for example, B10.A responds at *K^k*, is *I-A^k*, *I-B^k*) against which CTL can be generated. This suggests, assuming T cell help is generated in the context of *I-A* or *I-B*, that help would be provided preferentially to those T cell clones recognising *K* or *D* molecules of the same haplotype as *I-A/I-B*.

As each of these transformed cell lines forms tumours in T cell-deficient mice (*nu/nu* and anti-thymocyte serum-treated) and most are rejected in immunologically competent mice (unpublished data), the mode of recognition of the SV40-TASA and generation of specific cytotoxic lymphocytes might prove to be basic to the understanding of the control of tumour growth.

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Table 2 Differential response to SV40-TASA at *H-2D^d*

Effector cells*	% Specific lysis of SV40-transformed target cells†			
(<i>K I SGD</i>) <i>ABJC</i>	B10.D2SV	B10SV	B10.BRSV	B10.A(5R)SV
B10.D2 (<i>ddddddddd</i>)‡	20	1	2	18
B10.A (<i>kkkkkkddd</i>)‡	2	ND	57	1
B10.A(5R) (<i>bbbkkddd</i>)‡	3	42	5	26

ND, not determined.

* See legend to Table 1, *E/T* ratio 10:1.

† The spontaneous ⁵¹Cr release was less than 19%.

‡ Indicates the *H-2* haplotype.

Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus

THE M-variant of encephalomyocarditis virus (EMC) attacks pancreatic β cells and produces a diabetes-like syndrome in certain inbred strains of mice^{1,2}. Studies based on crosses between strains of mice that develop diabetes (susceptible) and strains of mice that do not develop diabetes (resistant) showed that susceptibility to EMC-induced diabetes is inherited as an autosomal recessive trait³⁻⁵. Although earlier experiments based on F₂ data had suggested that more than one genetic locus might be involved, lack of backcross data, wide variation between experiments, and the influence of non-genetic factors

on glucose levels precluded more precise genetic analysis⁵. The present investigation was initiated to study in greater depth the mode of inheritance of EMC-induced diabetes. The data presented here on susceptible and resistant parental strains and the F_1 and backcross offspring are consistent with a mendelian mode of inheritance, principally controlled by a single locus, involving two or more alleles.

Six-to-eight week old mice were infected with 1.0×10^4 plaque-forming units (PFU) of the M-variant of EMC virus that had been passaged in mouse hearts^{6,7}. The concentration of glucose in the blood was determined enzymatically by the glucose oxidase method⁸. Blood for glucose tolerance tests was obtained 60 min after intraperitoneal injection of 2 mg glucose per g body weight. Nonfasting (NF) glucose levels were measured 7 and 14 d after infection and the 60-min glucose tolerance tests (GTT) were carried out 10 and 17 d after infection. The data obtained on these four days were used to calculate the glucose index (GI) (ref. 5) for each mouse: $GI = ((4 \times NF \text{ day } 14) + (3 \times NF \text{ day } 7) + (2 \times GTT \text{ day } 17) + (1 \times GTT \text{ day } 10))/10$. An animal was scored as diabetic if its GI was equal to or greater than 3 standard deviations (s.d.) above the mean of the pooled data of uninfected mice.

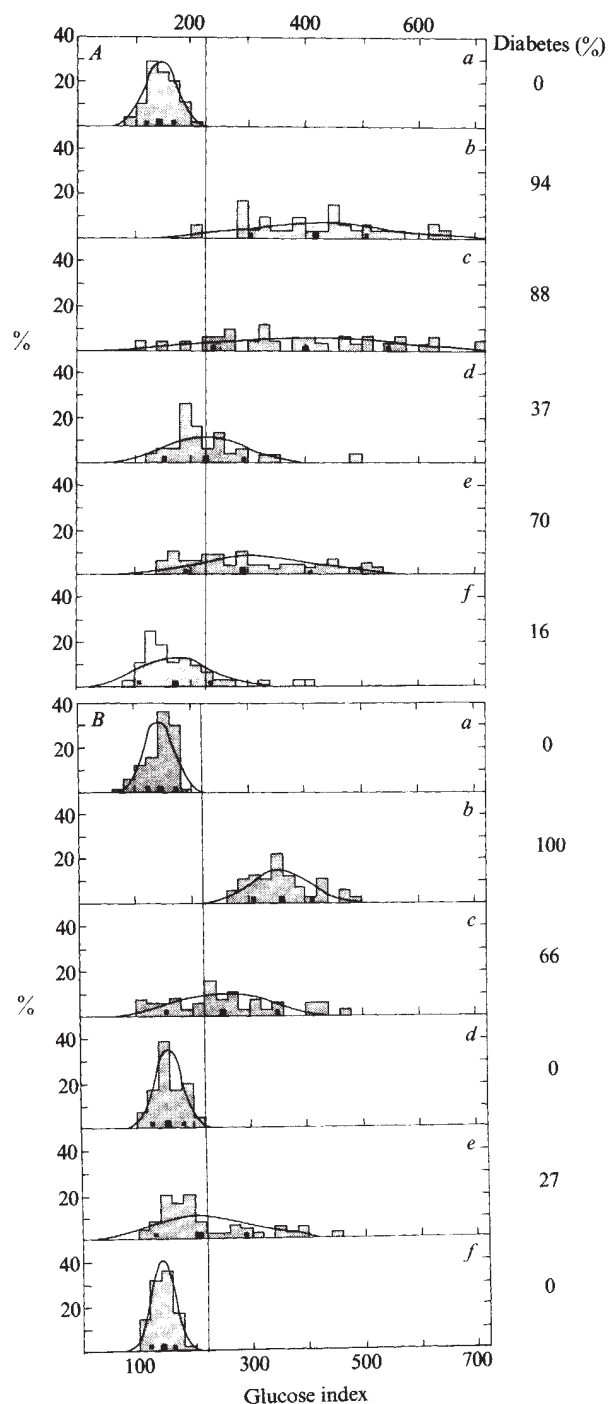
The GIs of all uninfected mice, pooled by sex, are plotted in Fig. 1 (*a* in *A* and *B*). The mean $\pm$ s.d. for males and females was 145 ± 27 and 143 ± 25 , respectively. When mice were infected with EMC virus, the GI became markedly elevated, with large differences occurring among strains and between sexes within strains (Fig. 1*A, B*). Male SJL and SWR mice had GIs of 420 ± 116 and 392 ± 152 , respectively. Approximately 90% of these animals were diabetic. The GIs of infected DBA/1 males (223 ± 69) were also elevated but not to the same degree as those of SJL and SWR males. Only 37% of the DBA/1 males had GIs in the diabetic range. When SJL and SWR mice were crossed and the F_1 hybrids infected, the male hybrids showed a GI pattern similar to that of the infected males of the parental strains, although both the mean GI (299 ± 110) and percentage of diabetic animals (70%) were lower. The offspring of the reciprocal crosses (that is, SJL $\times$ SWR and SWR $\times$ SJL) showed no difference in their mean GI. When SJL and DBA/1 mice were crossed and the male F_1 hybrids infected, the hybrids showed a glucose pattern (173 ± 65) more like that of infected DBA/1 than SJL males. Only 16% of the F_1 male hybrids were diabetic.

When females of these strains were infected with EMC virus (Fig. 1*B*), hyperglycaemia developed, but it was always less severe than that observed in male mice. In fact, the DBA/1 female mice did not become hyperglycaemic. When the (SJL $\times$ SWR) F_1 female hybrids were infected with EMC virus they showed a glucose pattern similar to that of the infected female parents, but as with the F_1 males, the mean GI and percentage of diabetic animals were lower than in either parent. The infected F_1 female offspring of SJL $\times$ DBA/1 failed to develop hyperglycaemia and the mean GI was more like that of infected DBA/1 than SJL females.

Figure 2 shows the GI of strains of male mice that did not develop hyperglycaemia after infection with EMC virus. Similar patterns were obtained when the females of these strains were infected (data not shown).

Figure 3 shows that the male F_1 hybrids resulting from the cross between susceptible SWR and resistant C57BL/6 mice were resistant to the development of EMC-induced diabetes. No significant difference was observed in the mean and variance of the reciprocal F_1 crosses. The mean GI of the F_1 males (174 ± 26), however, was slightly higher than that of the resistant C57BL/6 parents (124 ± 35). When the F_1 hybrids were crossed with the resistant C57BL/6 parents, the backcross males were found to be essentially resistant to the development of EMC-induced diabetes. When the F_1 hybrids were crossed with the susceptible SWR parents, the GIs of the infected backcross males showed a highly skewed distribution pattern with a long tail of high values similar to those in SWR mice and a peak in the normal range similar to that of the F_1 hybrids.

Fig. 1 GIs of susceptible strains of mice and their F_1 offspring. Mice were infected with EMC virus, GIs were determined, and the percentage of animals falling within each 20-unit interval was plotted (shaded areas). The small bars represent the mean $\pm$ s.d. The curves represent the expected distribution of the data based on the assumption that each population is normally distributed. The GI of uninfected mice of all strains (parental, F_1 , F_2 and backcrosses) were pooled and plotted at the top of each panel. An animal was scored as diabetic if its GI was 3 s.d. above the mean of the uninfected animals (vertical line). The first parent in each cross is the female. The F_1 hybrids include pooled data of reciprocal crosses (that is, (SJL $\times$ SWR) F_1 and (SWR $\times$ SJL) F_1). Numbers in parentheses represent number of mice in *A* (males) and *B* (females), respectively. *a*, Uninfected (346, 145); *b*, SJL(33, 40); *c*, SWR(33, 38); *d*, DBA/1(30, 31); *e*, (SJL $\times$ SWR) F_1 (49, 52); *f*, (SJL $\times$ DBA/1) F_1 (50, 51).



The present experiments, taken together with earlier data⁵, clearly show that susceptibility to EMC-induced diabetes is inherited as a recessive trait. This is supported by two types of observations. First, when susceptible strains (for example, SWR) are mated with resistant strains (for example, C57BL/6), the infected F₁ hybrids do not develop diabetes. Second, when susceptible SWR mice are mated with the less susceptible DBA/1 mice, the GIs of the infected F₁ hybrids are similar to those of the less susceptible DBA/1 parents. In addition, the present study shows that when two highly susceptible strains (that is, SWR and SJL) are crossed, the F₁ hybrids develop diabetes. As susceptibility is recessive, the development of virus-induced diabetes in the F₁ hybrids supports the argument that the same locus is of major importance in controlling susceptibility in both parental strains. The F₁ offspring of these susceptible strains, however, developed less severe diabetes than either parental strain.

Analysis of our data showed that the GIs of uninfected hybrids (males, 130 ± 25 ; females, 115 ± 17) were lower than those of the uninfected parental strains (males, 152 ± 9 ; females, 157 ± 6). Thus, the less severe hyperglycaemia in the infected hybrids as compared to the parental strains may not be related to a difference in viral susceptibility but instead may be due to modifier genes ('hybrid vigour'), particularly those involved in the regulation of blood glucose levels (for example, hormones, β cell regeneration). In this connection, it must be remembered that the elevated blood glucose levels in EMC-infected mice represent a response one or more steps removed from the initial interaction of the virus with β cells¹. Thus, a variety of genetic factors may play a secondary part in modulating glucose levels.

Support for the hypothesis that susceptibility to EMC-induced diabetes is primarily controlled by a single locus comes from backcross data. If the GI of the infected animal is under the control of a single autosomal locus, then half of the F₁ × C57BL/6 backcrosses would be expected to have the genotype of the F₁ hybrids and half the genotype of the C57BL/6 parents. In the case of the F₁ × SWR backcrosses, half would be expected to have the genotype of the F₁ hybrids

Fig. 2 GI of resistant strains of mice and their F₁ offspring. Data are from male mice. See legend to Fig. 1. *a*, AKR(18); *b*, A/J(12); *c*, C57BL/6(33); *d*, (AKR × A/J)F₁ (20); *e*, (AKR × C57BL/6)F₁ (14).

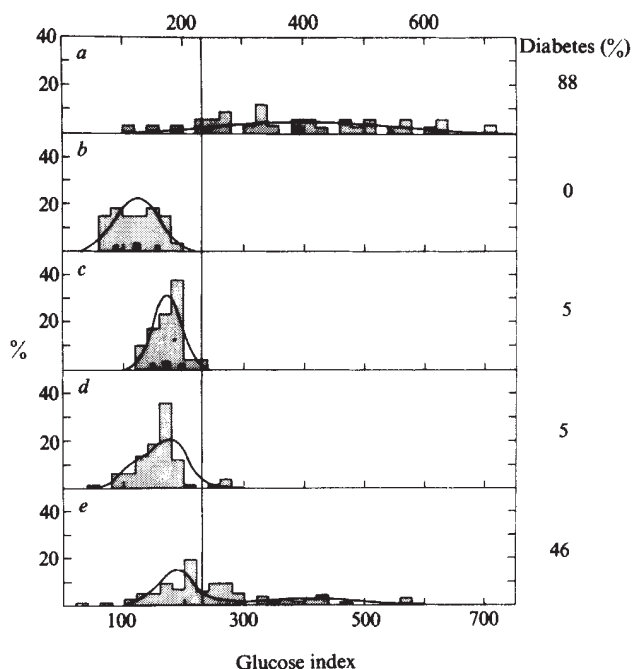
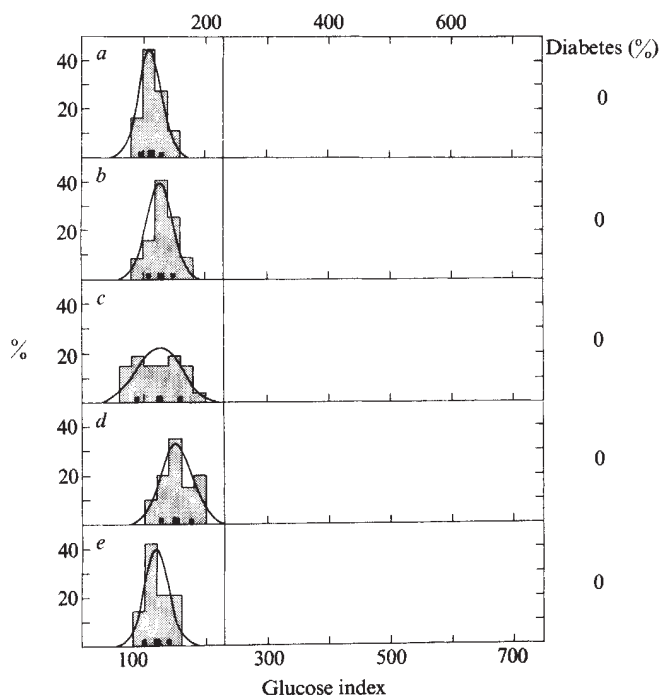


Fig. 3 GI of F₁ hybrids and backcrosses. (SWR × C57BL/6)F₁ hybrids were crossed with the susceptible (SWR) and the resistant (C57BL/6) parents. The curves in *a*–*c* were plotted on the basis of the actual data, assuming a normal distribution curve. The curves in *d* and *e* represent the expected distribution based on a one-locus model calculated from the mean and s.d. of the data in *b*, *c* and *a*, *c*, respectively. Data are from male mice. See text and legend to Fig. 1. *a*, SWR(33); *b*, C57BL/6(33); *c*, (SWR × C57BL/6)F₁(39); *d*, F₁ × C57BL/6(66); *e*, F₁ × SWR (93).

and half that of the SWR parents. Assuming a one-locus model, and that the males of inbred strains and their F₁ offspring represent genetically homogeneous populations with normally distributed GIs, an expected distribution curve based on the observed GIs of infected animals can be calculated. The curves in Fig. 3*d* and *e* show that the actual backcross data match the expected distribution curves quite closely. By χ^2 analysis of the observed frequency of diabetic animals in the F₁ × C57BL/6 and F₁ × SWR backcrosses, the hypothesis that susceptibility to the diabetogenic effect of EMC virus is controlled at a single locus was not rejected ($P > 0.05$).

In related experiments, up to 40% of the males and 12% of the females in the F₂ generation (that is, (SJL × SWR)F₁ intercross and (SJL × DBA/1)F₁ intercross) developed diabetes when infected with EMC virus (data not shown). The size of the groups was small and there seemed to be an excess of GIs within the normal range, but the data fit the single-locus model better than other models. Moreover, the dominance of the less susceptible DBA/1 genotype over the more susceptible SJL genotype, the resistance produced by the C57BL/6 genotype, and the differences among inbred strains of mice in the severity and percentage of animals developing hyperglycaemia suggest that there may be more than one allele controlling the degree of susceptibility. In addition, by determining the number of infected β cells with fluorescein-labelled anti-EMC antibody, we find that some β cells are infected even in resistant strains and that quantitative differences exist among the various strains^{9,10}. Thus, there seems to be a spectrum of susceptibility ranging from strains of mice showing only a few infected β cells and no hyperglycaemia to strains showing many infected β cells and hyperglycaemia. Although many complex genetic interactions might account for these various findings, the simplest explanation is a single locus involving two or more alleles.

The gene product controlling the development of virus-induced diabetes is still not known, but experiments suggest (but have not proved) that there might be more receptors for

EMC virus on the surface of β cells from strains of mice that develop diabetes than from strains that do not develop diabetes¹¹. If this is found to be the case, then it is possible that the locus controlling susceptibility may actually operate by modulating the expression of viral receptors.

The Jackson Laboratory sublines of SWR, SJL, DBA/1, C57BL/6, A/J, and AKR mice and the F₁, F₂ and backcross mice were either purchased or bred in our facilities from parental stock obtained from Jackson Laboratory, Bar Harbor, Maine.

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Epidermal growth factor, like phorbol esters, induces plasminogen activator in HeLa cells

STUDIES on the action of the tumour-promoting compound 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), and related plant diterpenes, in cell culture systems have revealed several unusual biological properties¹⁻³. These include the induction of plasminogen activator production² as well as several other phenotypic changes resembling those seen in cells transformed by chemical carcinogens or viruses¹. Furthermore, these compounds are extremely potent inhibitors of several types of terminal differentiation^{1,4,5}, all these effects being exerted by very low concentrations of the active compounds, in the range of 10^{-9} M. In addition, the activities of a series of such compounds, with respect to their cell culture effects, in general parallel their activities as promoters in the mouse skin two-stage carcinogenesis system^{1,6}. We have previously postulated that the highly pleiotropic effects of these compounds may be due to their ability to usurp the effector system of an endogenous growth regulatory hormone^{1,3,7,8}. The polypeptide hormone epidermal growth factor (EGF) is a possible candidate for this putative substance as it shares several biological properties with the phorbol esters⁹⁻¹⁴. Consistent with this hypothesis are recent results from our laboratory indicating that TPA is a potent inhibitor of the binding of ¹²⁵I-EGF to its cellular receptors³. We report here that EGF and TPA resemble each other in their ability to serve as potent inducers of plasminogen activator production in HeLa cell cultures. Our results further suggest that the mechanism of EGF induction of this protease is also similar to that of induction produced by TPA and related tumour promoters.

Plasminogen activator was assayed by methods reported elsewhere^{15,16}, and specific details are given in the legends to Figs 1 and 2. A cell lysate was used to measure total cell-associated activity, and extracellular activity was assayed in serum-free medium collected from cell cultures. Lysis of ¹²⁵I-labelled fibrin by cell samples was completely dependent on the

presence of plasminogen, indicating that we were dealing with a plasminogen activator. EGF or TPA added directly to the assay rather than to cells did not cause fibrinolysis.

Figure 1 shows the effect of increasing concentrations of EGF on cell-associated plasminogen activator in HeLa cultures. Induction was detected at a concentration of EGF as low as 1.25 ng ml^{-1} (2×10^{-10} M) and was maximal at 10 ng ml^{-1} (1.7×10^{-9} M). The induced levels were 7–12 times those present in untreated cultures. The time courses of induction of plasminogen activator in cell lysates and in media collected from HeLa cultures are shown in Fig. 2a and b. An increase in cell-associated plasminogen activator was observed as early as 4 h after the addition of EGF and continued to rise during the next 24 h (Fig. 2a). This was paralleled by a marked increase in plasminogen activator in the media of the HeLa cell cultures, presumably reflecting increased secretion of the protease. Actinomycin D ($10 \mu\text{g ml}^{-1}$) and cycloheximide ($100 \mu\text{g ml}^{-1}$) completely blocked EGF induction of plasminogen activator (Table 1), indicating that the induction requires both RNA and protein synthesis. These results, and the fact that both cell-associated and extracellular levels of plasminogen activator are increased (Fig. 2), provide evidence that EGF causes an increase in *de novo* synthesis of plasminogen activator. When TPA and EGF were tested in combination, at concentrations (5 ng ml^{-1} of each) which were suboptimal for each agent, an additive effect on plasminogen activator production was observed. When TPA (10 ng ml^{-1}) was tested in combination with a dose (10 ng ml^{-1}) of EGF which produced maximum induction of plasminogen activator, there was no augmentation or inhibition of the induction (Table 1). Phorbol, which lacks tumour-promoting activity and does not induce plasminogen activator^{2,7,8}, did not enhance or inhibit EGF induction of plasminogen activator, even when tested at 100 ng ml^{-1} (Table 1).

At present, the implications of these findings in terms of the mechanism of action of EGF are unclear. Our results do not apply to all cell types, for thus far we have not detected significant induction of plasminogen activator by murine EGF in chick embryo fibroblasts, a Chinese hamster cell line (CHO), a rat hepatoma cell line (HTC) or 3T3 mouse fibroblasts, even though the latter cell line is responsive to the growth stimula-

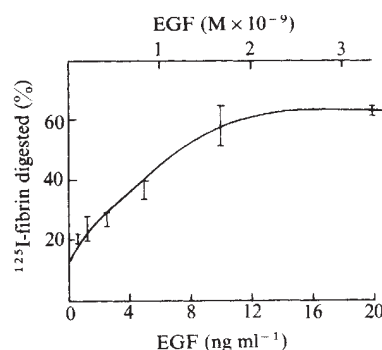


Fig. 1 Dose-response study of plasminogen activator induction by EGF in HeLa cells. Subconfluent cultures of HeLa, approximately 6×10^6 cells per 9-cm dish, were exposed to various concentrations of EGF in Eagle's minimum medium (MEM). After 18 h the cells were washed twice with phosphate-buffered saline (PBS) and collected into 2 ml of 10 mM Tris-Cl (pH 8), 10 mM NaCl and 0.5% Triton X-100. Aliquots containing 0.01 A₂₈₀ units were added, in triplicate assays, to ¹²⁵I-fibrin-coated plates in the presence of $5 \mu\text{g ml}^{-1}$ human plasminogen in assay buffer containing 0.1 M Tris-Cl (pH 8) and 10 mM NaCl. After a 1.5-h incubation at 37 °C, the solubilised ¹²⁵I-fibrin was collected and radioactivity was determined in a Searle Analytic 92 liquid scintillation counter programmed for ¹²⁵I. Percentage of fibrin digested was determined, using a trypsin-treated fibrin plate as the 100% value. The background, which was determined by omitting the sample, was subtracted from all assays.

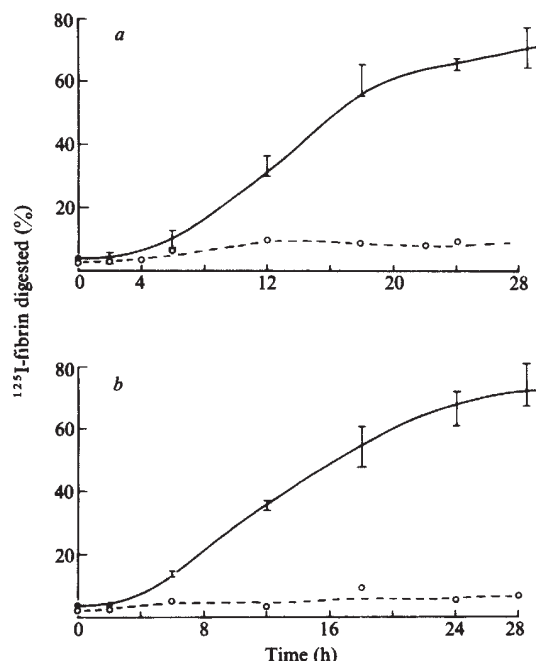


Fig. 2 Time course of induction of plasminogen activator by EGF. *a*, Cell lysate assay. EGF in MEM was added to culture plates of HeLa at zero time, and at the subsequent times indicated, cell lysates were prepared and assayed as in Fig. 1. *b*, Cell medium assay. Instead of cell lysates, the medium of the cells exposed to EGF was collected at the indicated times and centrifuged to remove any detached cells. The supernatant fluid (0.01 A_{280} units) was then assayed for plasminogen activator as described for cell lysates in Fig. 1. ---, Untreated HeLa; —, HeLa treated with 20 ng ml⁻¹ EGF.

tory effects of EGF¹⁷. The analysis of 3T3 is complicated, however, by the fact that even untreated cultures contain an appreciable level of plasminogen activator. EGF has growth stimulating effects on HeLa and other human cell cultures¹⁸. There is evidence that this stimulation requires an initial binding of EGF to cells, following which there is proteolysis of the bound EGF and an apparent decrease in EGF receptors¹⁸. Our results raise the possibility that these effects may be related to EGF induction of plasminogen activator or possibly other cell-associated proteases.

Table 1 Effects of various reagents on the production of plasminogen activator in HeLa cells

Reagents	Plasminogen activator (% fibrinolysis)
None	12
EGF (10 ng ml ⁻¹)	73
EGF (10 ng ml ⁻¹) + actinomycin D (10 µg ml ⁻¹)	13
EGF (10 ng ml ⁻¹) + cyclohexamide (100 µg ml ⁻¹)	2
EGF (5 ng ml ⁻¹)	37
TPA (5 ng ml ⁻¹)	42
EGF (5 ng ml ⁻¹) + TPA (5 ng ml ⁻¹)	75
TPA (10 ng ml ⁻¹)	88
EGF (10 ng ml ⁻¹) + TPA (10 ng ml ⁻¹)	90
Phorbol (100 ng ml ⁻¹)	8
Phorbol (100 ng ml ⁻¹) + EGF (5 ng ml ⁻¹)	36

Fibrinolysis assays were carried out on cell lysates using freshly prepared ¹²⁵I-fibrin plates, as described in Fig. 1, except that 8 µg ml⁻¹ of human plasminogen was used for each assay and the extent of fibrinolysis was measured after a 3-h incubation.

Several aspects of the induction of plasminogen activator by EGF resemble those previously described with TPA and related compounds^{2,6}. Both classes of agents act in the range 10⁻⁸–10⁻¹⁰ M and lead to an increase in both cell-associated and extracellular plasminogen activator. They also display similar time courses of induction and with both classes of agents the induction process requires RNA and protein synthesis. As mentioned above, EGF and TPA also share several other biological effects^{7–14}. These include growth stimulation, enhancement of sugar transport, induction of ornithine decarboxylase and prostaglandin synthesis, and promotion of mouse skin carcinogenesis. Further studies are required to determine the extent to which these similar effects reflect similar biochemical mechanisms of action and to elucidate the significance of such effects in terms of the processes of growth control and tumour promotion.

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Gradients in tumour growth

WHEN tumour cells are inoculated into syngeneic mice their establishment and growth is subject to regulation by the host animal. We have shown this most effectively for the inoculation of a broad range of tumours into anterior portions of the trunk¹. For example, when mammary tumour cells were injected intradermally into thoracic compared with lumbar regions of adult histocompatible mice, tumour growth anteriorly became detectable by more rapid palpation, and tumour growth was three to four times as rapid anteriorly as posteriorly. This difference between anterior and posterior injection sites also became manifest after subcutaneous inoculation; it was apparent after inoculation of a wide variety of tumour types (mastocytoma, melanoma, lymphoma, sarcoma, mammary adenocarcinoma); it was independent of the sex of the host animal; it was found in a variety of mouse strains; and it did not seem to be related to the immunocompetence of the host or to the immunogenicity of the tumour^{1,7}. We have now determined that there are antero-posterior and dorso-ventral differences in the growth of tumour cells inoculated intradermally into adult histocompatible mice.

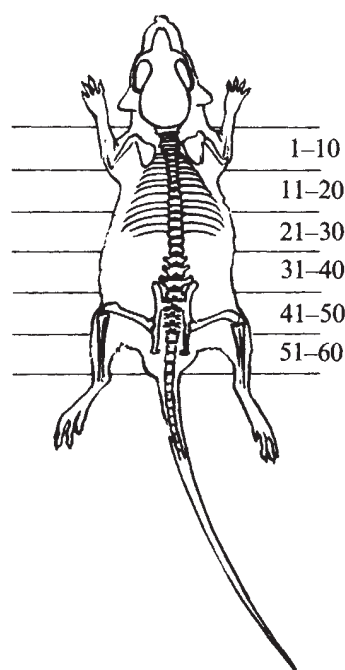


Fig. 1 Drawing of mouse skeleton, indicating the anatomical reference points used to characterise regional tumour growth. Numbers refer to distance (mm) from base of skull for an average 8-week-old C57BL/6 mouse.

Although we have no explanation for our results we suggest that the observed differences may reflect the existence of morphogenetic gradients reminiscent of those invoked to explain patterns of differentiation during ontogeny.

It seemed crucial to us to determine more precisely the topographical pattern of tumour growth, as this might provide some clue to the basis of the dramatic differences in the host animal's supportiveness for the growth and development of transplanted tumour cells. In our experiments we used the C755 mammary adenocarcinoma of C57BL/6 origin transplanted by the intradermal inoculation of 5×10^6 tumour cells in 0.1 ml saline into adult, syngeneic female mice. We have, however, carried out additional experiments with the S180 sarcoma inoculated into syngeneic BALB/C mice, with comparable results.

To determine the antero-posterior pattern of growth, tumour cells were injected into each of several points (three for any individual animal) along the middorsal line between the first vertebra and the sacrum. After an appropriate period (7–10 d) animals were killed and the tumours were excised and weighed. An inoculation site coordinate was specified on the basis of distance (mm) from the base of the skull to the beginning of the tail (60 mm for an average C57BL mouse), and normalised to permit comparison between experiments. Correlation of distance with actual location on the skeleton was also carried out to validate the normalisation procedures and to provide an anatomical reference point. For purposes of presentation and statistical analysis the neck and trunk were divided into six segments of equal length corresponding to the anatomical regions shown in Fig. 1.

We carried out four separate experiments involving a total of 196 tumour inoculations. All experiments gave comparable distribution patterns as validated statistically; they have therefore been combined for presentation in Fig. 2. It can be seen that there is a distinct pattern of tumour growth, with the peak in the thoracic region, declining anteriorly from this point ($P < 0.01$) and declining markedly as more posterior levels of the trunk were assayed ($P < 0.001$).

We have also tried to determine whether there was a dorso-ventral pattern of host supportiveness of tumour growth. We

Table 1 Relative growth of tumours inoculated dorsally, laterally or ventrally at various levels of the trunk

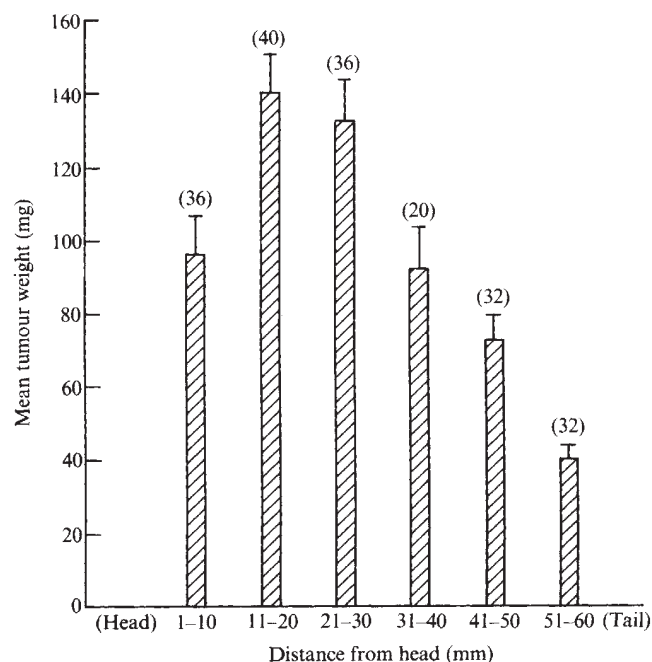
	Tumour weight (% $\pm$ s.e.)		
	Anterior	Middle	Posterior
Right lateral	17.8 $\pm$ 1.0	10.2 $\pm$ 0.9	3.8 $\pm$ 1.2
Left lateral	16.8 $\pm$ 1.2	7.1 $\pm$ 1.3	3.2 $\pm$ 0.8
Middorsal	12.7 $\pm$ 0.8	6.8 $\pm$ 1.8	3.6 $\pm$ 0.7
Midventral	5.9 $\pm$ 1.7	6.1 $\pm$ 0.8	6.0 $\pm$ 0.3

Antero-posterior differentials exist dorsally and laterally ($P < 0.001$) but not ventrally. Midventral growth rate significantly lower anteriorly ($P < 0.001$) and significantly higher posteriorly ($P < 0.001$), as determined using Student's *t* test. $n = 24$ for each value.

therefore chose three trunk levels (anterior, middle and posterior) at points approximately 15, 30 and 45 mm from the base of the head (see Fig. 1), and at each level we inoculated tumour cells in four sites (middorsal, right midlateral, left midlateral, and midventral). Each animal received 12 inoculations, and we carried out three separate experiments, each with eight animals. As the pattern observed was similar in each of the experiments, the data (288 tumour weights) were normalised and combined for presentation in Table 1. The experiments show that there is both an antero-posterior and a dorso-ventral pattern of tumour growth, and that the antero-posterior differential though present dorsally and laterally, is lacking in the ventral midline.

What we have tacitly assumed in the past is that the trunk is reasonably uniform, and that it would not matter precisely where in the trunk we should choose to inoculate cells as long as we were uniform in the type of inoculation (for example, intradermal or subcutaneous). This is certainly not so, inasmuch as we have observed a major difference in tumour size between tumours growing dorsally or laterally in the thoracic compared with the lumbar region, and between tumours permitted to develop at various positions in the thoracic level of the body. These differences must be borne in mind in future studies involving chemotherapy, immunotherapy or carcinogenesis², as the base rate of tumour growth (control level) is perhaps the single most critical value in the

Fig. 2 Regional differences in the growth of C755 mammary carcinoma cells inoculated intradermally at various levels of the middorsal trunk region of adult syngeneic mice. Figures in parentheses represent the number of tumours assayed in each group.



design and interpretation of such studies. Examination of the low s.e. values in our experiments (Fig. 3) suggests, moreover, that much of what we have previously ascribed to 'normal' variability in tumour growth may be averted by a more rigorous definition of injection protocols.

It is fascinating to speculate on the biological basis underlying the observed topographical pattern of tumour development. The developmental biologists long ago recognised the existence of both antero-posterior and dorso-ventral gradients operative during ontogeny, and it would seem reasonable to suggest that such gradients may still exist even in the adult. Such a suggestion is supported by a number of related observations: a differential rate of DNA synthesis has been observed in the epidermis after wounding, the higher rate being found anteriorly than posteriorly³. (A dorso-ventral differential was not considered in these studies.) A preference for tumour production in response to carcinogens to occur anteriorly has been found to exist for mice implanted with methyl cholanthrene-containing pellets². Control factors operating during embryogeny have been shown to continue to be relevant to organ maintenance in adults⁴; and there are numerous suggestions of other complex developmental patterns persisting into adult life, of which those involving the vascular system and the nervous system would seem to be the most immediately provocative ones^{5,6}.

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Ethanol-induced tolerance to heat and to adriamycin

HEAT can induce a transient state of thermal tolerance so that mammalian cells surviving one exposure to hyperthermia are much more resistant to a subsequent heat exposure^{1,2}. Studies of the combined and sequential cytotoxic effects of hyperthermia and adriamycin (ADM) show that cells exposed to heat could then also develop considerable resistance to ADM³. The most reasonable explanation for the latter finding is that prolonged heat exposure modifies the cell membrane permeability to ADM. No precise mechanisms were postulated as being responsible for either type of tolerance. We report here that ethanol induces tolerance to heat and also tolerance to ADM. The qualitative patterns of cellular survival closely resemble those induced by heat exposure. These results reinforce the hypothesis that one of the prime targets of inactivation of mammalian cells by heat is the cellular membrane.

Recently, work from our laboratory has also shown that aliphatic alcohols inactivate mammalian cells; the alcohols with longer carbon chains are more effective⁴. The shape of the survival curves and the relative potency of the alcohols is remarkably similar to the published data of a spin label study on alcohol-induced changes in membrane fluidity⁵. Elevated

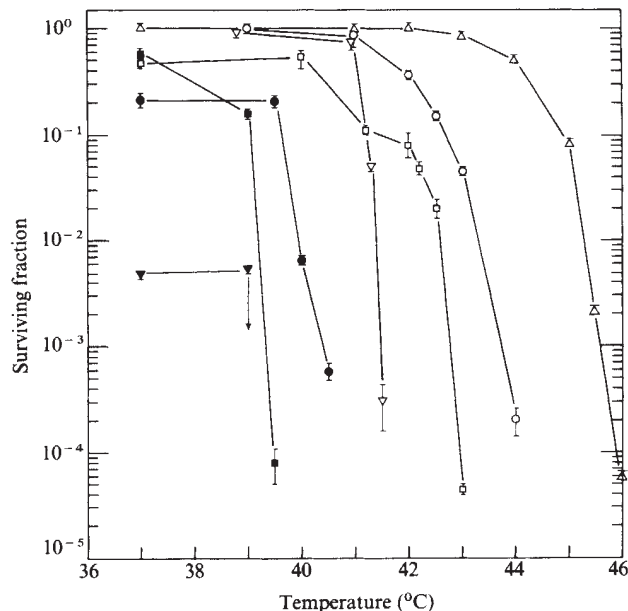


Fig. 1 Survival of Chinese hamster cells exposed to ethanol (0-1.1 M) for 30 min at various temperatures. Note that the presence of ethanol lowered the temperature threshold above which cells were rapidly inactivated. ▽, 1.12 M; ■, 0.97 M; ●, 0.82 M; ▽, 0.66 M; □, 0.50 M; ○, 0.33 M; △, 0 M.

temperatures reduce the amount of alcohol required to kill cells and vice versa⁴. The similarity between the cellular responses to these two agents lead to the hypothesis that excessive membrane fluidity results in cellular inactivation, whether this fluidity is induced by alcohols or hyperthermia, or by a combination of the two. If the mechanism of cytotoxicity by heat and by alcohols is similar, then it seems reasonable to suggest that induction of tolerance is also similar and that exposure of cells to ethanol (at 37°C) might make them more resistant to a subsequent exposure of either heat or ADM.

Monolayers of Chinese hamster cells of ovarian origin, HA-1, were cultivated in routine conditions^{6,7} at 37°C. Exponentially growing cells were used for all the experiments and the plating efficiencies were 70-90%. For thermal exposure, the temperature of the specially designed water bath⁸ was controlled to within 0.1°C, and the pH of the medium regulated by a constant flow of 95% air and 5% CO₂ to values between 7.0 and 7.4. In all experiments, media were exchanged just before exposure of cells to either heat or drug. Immediately after each exposure the cells were rinsed at least twice with phosphate-buffered saline. The assay for cellular survival was the cloning technique developed by Puck and Marcus⁹.

Results of experiments in which cells at fixed ethanol concentrations were exposed to various temperatures (37-45°C) are shown in Fig. 1. When survival curves were plotted against temperature, with ethanol concentration as a fixed parameter, the presence of small amounts of ethanol during heating was seen to lower the temperature threshold required to inactivate cells. In other words, elevated temperatures facilitated cell inactivation by ethanol. This was indicated by the reduced temperature required in the presence of ethanol to achieve a given level of cell killing; the higher the ethanol concentration, the lower the temperature needed. These results confirm and extend our earlier findings⁴.

To test for the induction of thermal tolerance, cells were exposed to 43°C for 1 h, or to 6% ethanol for 1 h at 37°C (their normal growth temperature). The temperature and duration of heating as well as ethanol exposures were chosen so that approximately 50% of cells survived in either case. For the ethanol group, 37°C was used so that the cells were not exposed to an elevated temperature before the second

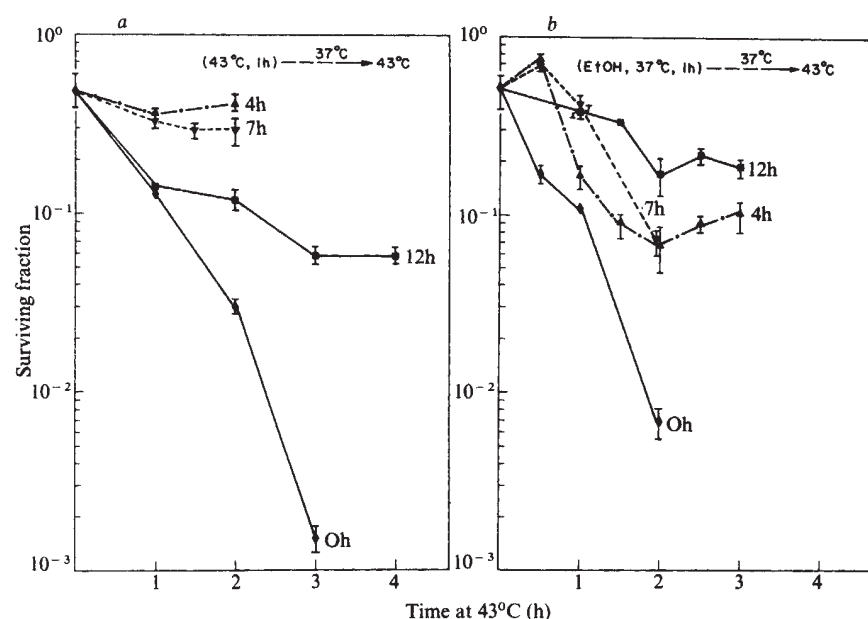


Fig. 2 Survival of Chinese hamster cells as a function of duration of exposure at 43°C given as the second challenge. *a*, Cells were initially exposed to 43°C for 1 h (first challenge); *b*, cells were initially exposed to 6% ethanol for 1 h at 37°C. Length of time at 37°C incubation between the two challenges is as indicated. The survival value of the initial exposure to heat or ethanol is indicated by the intersection of the ordinate and the 0 h curves.

challenge. After the initial treatment, some of the cells were immediately heated to 43°C for graded periods of time. The others were returned to the 37°C incubator for various lengths of time before similar second challenges. The survival curves obtained, plotted as a function of duration of the second challenge, are shown in Fig. 2. After the initial exposure to either heat or ethanol, the cells clearly acquired a tolerance to the subsequent heat challenge as evidenced both by the increase in survival values and the reduced slope of the survival curves. The degree of the induced tolerance was a function of the time interval at 37°C between the two exposures. Although there are quantitative differences, the patterns of heat or ethanol-induced thermal tolerance are remarkably similar. We then carried out another set of experiments in which the second challenge consisted of graded doses of ADM (0–10 $\mu\text{g ml}^{-1}$). When the survival curves so obtained were plotted as a function of ADM concentration (Fig. 3), we again found that not only heat but also ethanol induced cellular resistance to

ADM. The qualitative patterns of heat- or ethanol-induced ADM tolerance are again quite similar.

The plasma membrane has been suggested as the primary target of heat-induced cellular inactivation¹⁰. Recent studies show that hyperthermia modifies membrane transport phenomena¹¹. The interaction of the polyene antibiotic amphotericin B and hyperthermia¹², the changes in membrane morphology after heat exposure^{13,14}, and the effects of the combined cytotoxicity of heat and ADM³, are all evidence in support of the membrane permeability hypothesis. Ethanol and other aliphatic alcohols have been demonstrated to modify the fluidity of membranes by introducing disorder into the lipid bilayer⁵. Studies of the interactions between hyperthermia and n-alcohols⁴ have shown that there is at least a measure of duality between the cytotoxic actions of alcohols and hyperthermia. We have shown here that exposure of cells to ethanol makes them much more resistant to a subsequent exposure of heat or ADM and that the patterns of cellular survivals of

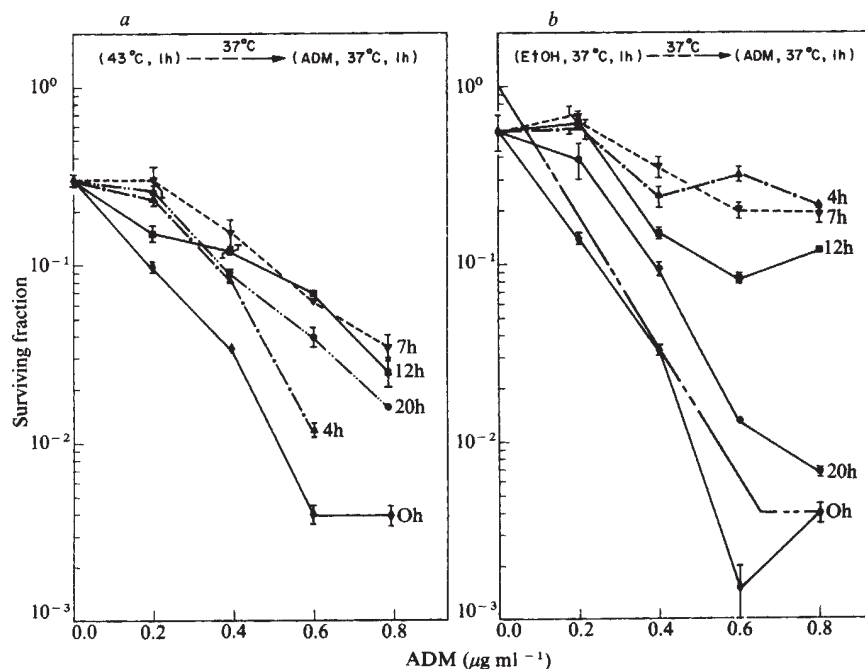


Fig. 3 Survival of Chinese hamster cells as a function of graded concentrations of ADM given as the second challenge. *a*, Cells were initially exposed to 43°C for 1 h; *b*, cells were initially exposed to 6% ethanol at 37°C for 1 h. Detailed experimental procedure as in Fig. 2. Survival values of cells exposed to graded doses of ADM at 37°C are indicated by the dash-dot-dot curve in *b*. The latter results are shown to demonstrate that it is resistance to ADM that develops, rather than the disappearance of a heat-induced sensitisation³.

ethanol-induced tolerances bear remarkable resemblance to those induced by an initial heat exposure. All these results strongly emphasise the similarity between the effects of heat and ethanol. Cellular inactivation by ethanol, as has been discussed earlier, is probably determined by membrane fluidity. Thus, hyperthermic inactivation is likely to be similarly determined. This view is clearly reinforced by the concanavalin A agglutination data, which show similar agglutination patterns after previous heat or ethanol exposures⁴.

Henle and Dethlefsen¹⁵ concluded that thermotolerance is correlated with a reduced inactivation entropy, as is also the case with a heat-resistant strain of pig kidney cells isolated by Harris¹⁶. Reduced entropy implies increased order, as would be expected in less fluid structures. Other studies have shown that an increase in the permeability of a number of different compounds is a consequence of increased membrane fluidity^{17,18}. The ADM tolerance we have observed induced by heat or ethanol in mammalian cells is probably related to permeability, and therefore may be a fluidity related phenomenon. However, both in the case of ethanol and of hyperthermia exposure, not only membranes but all other cellular organelles are affected. The correlations shown here as well as the other results cited, suggest that the membrane is involved and that fluidity plays a role. Direct measurements of membrane fluidity after both heat and ethanol exposures are currently underway, and these should unequivocally determine whether or not membrane fluidity and thermal sensitivity are necessarily correlated.

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Cellular mechanism for the protective effect of haemoglobin S against *P. falciparum* malaria

RELATIVE protection against *Plasmodium falciparum* malaria afforded to heterozygous carriers of the sickle-cell gene is now the accepted mechanism for the high frequency of the gene in areas where malaria is (or was) common^{1–3}. However, the cellular mechanisms whereby Hb S protects the red cell against the malarial parasite are still not fully understood. We have compared the rates of invasion and growth of *P. falciparum* in normal red cells and in those of individuals with the sickling disorders, in both aerobic conditions and conditions of reduced oxygen tension. As a result of these studies, we suggest a possible mechanism for the protection of sickle-cell heterozygotes against *P. falciparum* malaria.

The rates of invasion by *P. falciparum* into AA (normal adults), AS (sickle-cell heterozygotes), and SS (sickle-cell homozygotes) cells maintained in either aerobic conditions or under reduced oxygen tension are compared in Table 1. In AS cells there was a slight reduction in the rate of invasion under low oxygen tension as compared with aerobic conditions, whereas the opposite effect was observed in AA cells. This difference in invasion rates at differing oxygen tensions

Table 1 Invasion by *P. falciparum* of red cells under aerobic conditions or low oxygen tension

A	AA			AS		
	Expt. no.	Aerobic	Low O ₂	Aerobic	Low O ₂	Low O ₂
			Aerobic			Aerobic
1	15.5	14.9	0.96	13.5	12.4	0.92
				16.5	13.2	0.80
2	10.3	10.5	1.02	13.4	12.7	0.95
				14.6	13.4	0.92
3	7.7	7.9	1.03	8.1	7.9	0.98
				8.6	7.2	0.84
4	5.9	6.9	1.17	6.4	6.1	0.95
	6.2	6.1	0.98	6.1	5.5	0.90
5	12.8	11.8	0.92	15.5	12.2	0.79
	10.8	13.0	1.20			
6	18.7	18.3	0.98	17.2	18.9	1.10
7	7.7	12.2	1.58	6.8	4.3	0.63
Mean ± s.e.m.			1.09 ± 0.07			0.89 ± 0.04
P			0.02 > P > 0.01			
B	AA			SS		
	Expt. no.	Aerobic	Low O ₂	Aerobic	Low O ₂	Low O ₂
			Aerobic			Aerobic
1	16.8	15.4	0.92	22.9	11.6	0.51
2	10.4	12.2	1.17	12.9	11.4	0.88
3	18.5	16.3	0.88	21.8	14.4	0.66
4	10.9	14.6	1.34	14.9	9.5	0.64
Mean ± s.e.m.			1.08 ± 0.11			0.67 ± 0.08
P			0.05 > P > 0.02			

A, Normal adults (AA) and sickle-cell heterozygotes (AS); B, normal adults (AA) and sickle-cell homozygotes (SS). The figures given are the numbers of parasites per 100 red cells after invasion of uninfected cells *in vitro*. In each case, at least 1,000 cells were counted. Samples of 5 ml of blood containing synchronous late ring stages of *P. falciparum* were collected into heparin (10 IU ml⁻¹). After removal of white cells, the red cells were maintained in culture until the parasites had reached the schizont stage, after which the schizont-infected cells were concentrated and then suspended in a mixture of 3 parts Medium 199 (Flow Laboratories) and one part non-immune AB serum^{4,5}. Uninfected red cells were obtained from normal adults, sickle-cell heterozygotes and sickle-cell homozygotes, washed three times in PBS and the buffy coat removed. They were then mixed with an appropriate volume of parasitised red-cell suspension, so that the final concentration was approximately 80×10^3 cells μl^{-1} with 2–5% schizont-infected cells. The mixture was pipetted into the wells of a microtissue culture plate (250 μl per well) and incubated either in 5% CO₂ in air or in an environment containing 5% (35 mm Hg) oxygen produced by gassing with a mixture of 2% oxygen–5% CO₂–93% nitrogen. The oxygen tension was measured with an oxygen electrode (Becton Dickinson) placed inside the culture vessel. The cultures were incubated at 37 °C without shaking. At various intervals (usually 12, 36 and 48 h) blood films were made from the cultures. Red cells containing parasites grown at low oxygen tension were also fixed in suspension by adding 50 μl of 40% buffered formaldehyde (pH 7.4) at 4 °C to the appropriate wells immediately after removal from the gassed environment. All smears were then fixed in methanol and stained with Giemsa. The rate of invasion was determined by examining at least 1,000 cells in smears taken from the cultures at about 12 h and counting the number of ring-form parasites per 100 red cells. Parasite growth was assessed at about 48 h by determining the maturity of parasites in 100 singly infected cells.

between AA and AS cells, although small, was significant ($0.02 > P > 0.01$). In SS cells there was a marked reduction in the rates of invasion under reduced oxygen tension which was significantly different from the increase in invasion observed in AA cells ($0.05 > P > 0.02$). Examination of formaldehyde-fixed smears prepared from cultures containing SS cells showed that the reduction in invasion had occurred to a similar degree in both sickled and non-sickled cells (Table 2). In cultures containing AS cells, parasites were seen in both sickled and non-sickled cells; since only very few of the cells had sickled however, the majority of parasites were in non-sickled cells. The decreased rate of invasion was thus not restricted to sickled forms.

Table 3 shows a comparison of the growth of *P. falciparum* in AA, AS and SS cells. In aerobic conditions there was a slight but consistent retardation of growth in both AS and SS cells compared with AA cells. In conditions of low oxygen tension, however, there was a striking retardation, and in some cases almost total inhibition, of parasite growth in both AS and SS cells. Although assessment of parasite development in formaldehyde-fixed smears was not possible, these preparations showed that in cultures containing AS cells the majority of parasites were present in non-sickled cells. For example, in the experiment shown in Table 3, 83% of the singly infected cells were not sickled and yet none of the parasites had developed beyond the stage of small rings. This indicates that sickling is not necessary for growth retardation in cells containing Hb S. This could not be further studied in the cultures of SS cells, since at the time of assessment of growth almost all the cells had sickled.

In an attempt to simulate *in vivo* conditions, parasites were cultured to the small trophozoite stage (corresponding to the time *in vivo* when they would be present in well oxygenated peripheral blood) and then placed into conditions of low oxygen tension (corresponding *in vivo* to the period when the parasites would have moved into the poorly oxygenated deep tissues such as spleen, liver, bone marrow and brain). After transfer from aerobic conditions to a 12-h incubation at low oxygen tension, blood smears were made and the parasite growth in AA and AS cells were compared. Growth of these more mature parasites was clearly retarded in the AS cells under conditions of low oxygen tension, although this did not occur in the AA cells (Table 4). Again, examination of formaldehyde-fixed preparations showed that the majority of singly infected cells were unsickled, indicating that growth retardation was independent of sickling.

These experiments demonstrate that, although there is minimal retardation of invasion and growth of *P. falciparum* in cells containing Hb S maintained aerobically, a marked reduction in both invasion and development occurs when these cells are exposed to conditions of low oxygen tension. It seems very likely that this effect is due to the properties of Hb S, because exposure of normal erythrocytes to reduced oxygen tension potentiated parasite invasion and growth if it had any effect at all. Furthermore, it is evident that to elicit the effects of reduced oxygen tension on the rates of invasion and growth of *P. falciparum* in cells containing Hb S, the cells need not sickle; a low oxygen tension is sufficient.

Table 2 Distribution of *P. falciparum* among sickled and non-sickled cells from a sickle-cell homozygote after invasion under low oxygen tension

	Aerobic	Low oxygen tension	
		Sickled cells (51%)	Non-sickled cells (49%)
Cells counted	1,921	1,912	1,804
Parasites counted	440	199	208
Parasites per 100 red cells	22.9	10.4	11.5

Table 3 Results of typical experiments and summary of all experiments on the growth of *P. falciparum* in normal adult red cells, sickle-cell heterozygotes and homozygotes

	AS				SS			
	Aerobic		Low O ₂		Aerobic		Low O ₂	
	AA	AS	AA	AS	AA	SS	AA	SS
Morphology:								
Small ring	0	0	0	26	0	0	0	32
Large ring	1	1	0	0	2	3	1	39
Small trophozoite	18	24	14	0	12	15	4	7
Large trophozoite	65	56	59	0	16	25	17	1
Immature schizont	10	15	15	0	14	10	10	1
Mature schizont	4	1	10	0	51	43	68	0
Abnormal form	2	3	2	74	5	4	0	20
Mean growth score	3.9	3.8	4.1	0.3	4.8	4.6	5.4	1.4
Difference	-0.1		-3.8		-0.2		-4.0	
Total no. of cultures	17		12		6		3	
Mean difference for all experiments	-0.2		-3.0		-0.3		-3.1	

In each culture, the maturity of parasites in each of 100 singly infected cells was assessed and given an arbitrary score from 1 to 6 according to the following classifications: small ring = small parasite $< 2 \mu\text{m}$ with no pigment—score 1; large ring = large parasite, approximate size $> 2 \mu\text{m}$ but no pigment—score 2; small trophozoite = first appearance of a single clump of pigment—score 3; large trophozoite = larger parasite with increased pigment but single nucleus—score 4; immature schizont = first evidence of nuclear division (two or three nuclei)—score 5; mature schizont = four or more nuclei—score 6; abnormal form = pyknic hyperchromatic parasite with irregular outline—score 0. The mean growth score (MGS) was derived by adding the scores of 100 parasites and dividing by 100. The difference is the MGS of the control cells (AA) subtracted from the MGS of the test cells.

There is good evidence that cells containing Hb S undergo significant physical changes when subjected to reduced oxygen tension before sickling is demonstrable⁶. Areas of electron-dense material become visible on electron microscopy and the cells become more rigid, as shown by their inability to pass through $3\text{-}\mu\text{m}$ pores in polycarbonate sieves. It seems likely that some of these physical alterations on exposure to low oxygen tensions could be responsible for the reduced rate of invasion and growth of *P. falciparum* in Hb S-containing cells.

These observations suggest a mechanism for the protection of sickle-cell heterozygotes from *P. falciparum* malaria *in vivo*. After development of schizonts inside the liver the liberated merozoites invade red cells. The resultant ring forms mature quite readily in well oxygenated peripheral blood where they normally spend the first 36 h of the erythrocyte cycle⁷. After this, the infected cells retreat to the deep tissues where the parasites mature to schizonts during the last 12 h of the cycle. During this period, the cells are exposed to oxygen tensions very similar to those which caused retardation of parasite development in the present experiments. Hence in red cells containing Hb S this stage of parasite maturation might be retarded. Furthermore, the inhibition of invasion of released merozoites into Hb S-containing cells under these low oxygen tensions would reduce the degree of parasite multiplication. If the parasitaemia were to increase, the resulting inflammation and blockage of the capillaries in the deep tissues would increase the degree of vascular stasis and anoxia⁸ and thus provide an even more unfavourable environment for parasites in cells containing Hb S.

Our proposed mechanism for the protection of sickle cell heterozygotes against *P. falciparum* has several attractions. It explains why protection is predominantly against *P. falciparum* malaria, since this is the only human malaria in which the

parasite is confined for at least part of its erythrocytic cycle to deep tissues where the oxygen tension is low. It also explains why individuals with sickle-cell trait remain susceptible to infection by *P. falciparum*, but how increasing parasitaemia works to the detriment of parasite survival. This would be in agreement with many epidemiological studies which have shown that sicklers can become infected but that high parasitaemia and subsequent mortality is rarer amongst such individuals^{9,10}. Furthermore, the mechanism does not rely on the sickling of AS cells to produce a detrimental effect on the parasites. This is important because it has always seemed unlikely that *in vivo* sickling occurs to any marked degree at physiological oxygen tensions in sickle-cell heterozygotes¹¹.

Unlike previous investigations, a deleterious effect of Hb S on parasites has been clearly demonstrated *in vitro*. In the experiments of Luzzatto *et al.*¹², parasitised AS cells were shown to sickle more rapidly than unparasitised control AS cells in 8% sodium metabisulphite, presumably because the parasites had used oxygen from the red cells. Luzzatto *et al.* suggested that this phenomenon might occur *in vivo* and that the parasitised sickled cells might be removed preferentially from the circulation and therefore the parasite could not complete its life cycle. It seems unlikely, however, that a parasite can cause sufficient reduction in oxygen tension in an AS cell to cause it to sickle and remain sickled in peripheral blood. A mechanism whereby the parasite's capability for invasion or growth in AS cells in areas of reduced oxygen tension is limited, seems a more likely basis for the protection afforded by AS cells.

Friedman¹³ has shown that malarial parasites maintained in continuous culture do not develop in AS or SS cells under conditions of reduced oxygen tension: however, he found that this effect was only demonstrable with young parasites and not with mature forms. This fails to explain why Hb S should protect against malaria, since these immature forms are found mainly in well oxygenated peripheral blood. In the present experiments it was found that growth retardation occurred in the more mature forms as well. It is difficult to understand the

discrepancy between the present findings and those of Friedman, but recent experiments indicate that in anaerobic jars, at least, the oxygen tension at a depth of 8 mm in a stationary medium may take up to 30 h to equilibrate with the surrounding gaseous environment. This phenomenon might explain the failure of Friedman to observe retardation of growth of mature parasites and also why in some cases in the present study invasion of AS cells under low oxygen tension was not markedly decreased.

These experiments demonstrate that at the cellular level Hb S has a detrimental effect on the proliferation of *P. falciparum*. This involves both invasion of the parasite into the red cell and growth once inside, and requires conditions of low oxygen tension. Actual sickling of the cells concerned is not necessary. These studies provide an explanation for the protection of sickle cell heterozygotes against *P. falciparum* malaria and thus for the high frequency of the sickle-cell gene in parts of the world where malaria is (or has been) endemic.

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Table 4 Growth of *P. falciparum* in red cells from sickle-cell heterozygotes where reinvasion occurred under aerobic conditions but the culture had been maintained under low oxygen tension after most of the parasites had reached trophozoite stage

Expt. no.	1			2		
	AA	AS	AS	AA	AA	AS
Cells	—	19.0	29.4	—	—	29.4
Hb S (%)	—	19.0	29.4	—	—	29.4
Sickled cells (%)	0	2	2	0	0	2
Morphology:						
Small ring	0	0	0	0	0	2
Large ring	2	2	8	7	0	8
Small trophozoite	28	28	50	18	16	27
Large trophozoite	27	37	13	33	32	24
Immature schizont	9	16	3	7	13	6
Mature schizont	24	7	3	30	27	12
Abnormal form	10	10	23	5	12	21
Mean growth score	3.9	3.4	2.5	4.2	4.0	3.0
Mean growth score (aerobic)	3.9	3.8	3.6	4.2	4.0	4.1
Difference	0	-0.4	-1.1	0	0	-1.1
Mean growth score (low O ₂)	4.1	0.3	0.1	4.9	4.0	0.1

The classification and scoring of parasites is as in the legend to Table 3. The difference is the MGS of a similar culture under aerobic conditions (MGS aerobic) subtracted from the MGS of the culture under conditions outlined above. The MGS under low oxygen tension (MGS low O₂) is also included for comparison.

Agonist regulation of the human platelet α -adrenergic receptor

PROLONGED exposure to drugs or hormones may result in diminished physiological responsiveness which is referred to as tolerance or desensitisation. One mechanism which has been proposed to explain tolerance is a change in the state of tissue receptors following activation by drugs or hormones¹. The receptor desensitisation hypothesis to explain tolerance to the β -adrenergic effects of catecholamines has been particularly well characterised in the frog erythrocyte¹⁻⁴. In contrast, the regulation of catecholamine α -adrenergic receptor-mediated responses has been much less extensively investigated. Although the development of rapid desensitisation or tachyphylaxis is not a prominent feature of many α -adrenergic responses, rat parotid cells lose their α -adrenergic mediated potassium secretory response after 2-4 min of (-)-adrenaline exposure⁵. Concomitantly, there was a decrease in binding of the α -adrenergic antagonist ³H-dihydroergocryptine to these cells which was thought to represent either a decrease in number or affinity of α -adrenergic receptors. It has been hypothesised that α - as well as β -adrenergic responses in man

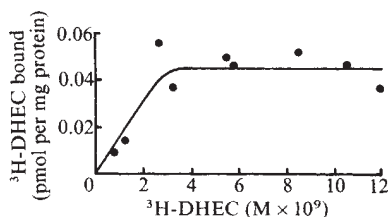


Fig. 1 Saturation of platelet binding sites with ^3H -dihydroergocryptine (DHEC). Platelet-rich plasma was obtained from blood anticoagulated with 13 mM sodium citrate by centrifugation at 160 g for 10 min. The platelet-rich plasma was made 2.5 mM in EDTA and the platelets sedimented at 2,500 g at 25°C for 10 min. The platelets were washed once and then resuspended in assay buffer [138 mM NaCl, 5 mM MgCl_2 , 1 mM EGTA, 25 mM Tris-HCl, pH 7.55 (Tris-saline)] at a protein concentration of 3–5 mg ml^{-1} . Increasing concentrations of ^3H -DHEC were added in the presence or absence of 10^{-4}M (–)adrenaline. Incubations were carried out for 20 min at 25°C in a total volume of 2 ml Tris-saline. The assay mixture contained 300–500 μg of platelet protein. After incubation 1.8 ml were added to 3 ml Tris-saline buffer at room temperature and rapidly filtered through a Whatman GF/C glass-fibre filter under reduced pressure. The assay tube and filter were then washed with four 5-ml aliquots of the Tris-saline buffer and then filter dried and counted in a liquid scintillation spectrometer. All binding is expressed as 'specific binding', the difference between radioactivity bound in the presence and absence of 10^{-4}M (–)adrenaline. Each point represents the mean of triplicate determinations. Specific binding ranged from 55% at the lowest concentrations of ^3H -DHEC to 18% at the highest concentration. At the highest concentration bound radioactivity ($\sim 9,000$ c.p.m.) represented 3.1% of added counts of which approximately 0.5% represented the filter blank. At saturation there were 0.045 pmol of ^3H -DHEC bound per mg protein.

diminish with chronic exposure to high concentrations of catecholamines⁶. The human platelet would seem to be a useful tissue to investigate this possibility, as catecholamine-induced platelet aggregation is mediated through α -adrenergic receptors. We have recently characterised the human platelet α -adrenergic receptor by ligand binding assay using ^3H -dihydroergocryptine and have shown good correlation between binding characteristics and the biochemical and physiological manifestations of α -adrenergic receptor activation⁷. We now report that the human platelet α -adrenergic receptor is subject to ligand regulation as assessed by ^3H -dihydroergocryptine binding and that this regulation is reflected in altered physiological function.

α -Adrenergic receptors in platelets were studied indirectly by measuring catecholamine mediated aggregation and release of ^{14}C -5 hydroxytryptamine (5-HT) and directly by ligand binding assay. Platelet aggregation was assessed using a standard nephelometric technique⁸ in which 0.4 ml aliquots of pla-

telet-rich plasma were stirred at 1,000 r.p.m. at 37°C in the presence of an aggregating agent such as adrenaline, ADP, or collagen. Platelet ^{14}C -5-HT release was measured by a modification of the method of Jerushalmy and Zucker⁹. After completion of aggregation induced release of ^{14}C -5-HT into the plasma, the reaction was stopped by adding EDTA (final concentration 17 mM) and cooling to 4°C. The samples were processed for liquid scintillation counting as previously described¹⁰.

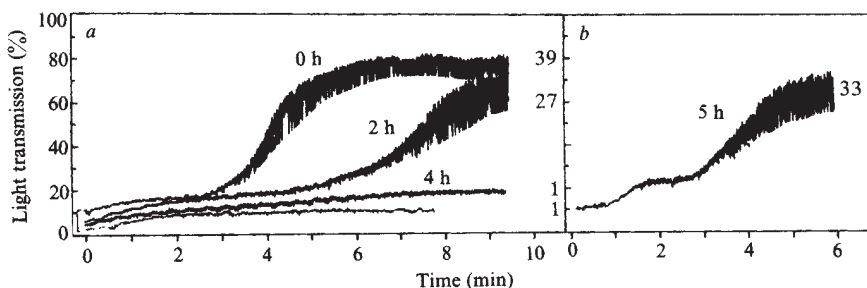
The platelet α -adrenergic receptor was assessed directly using ^3H -dihydroergocryptine (24.1 Ci mmol^{-1} , NEN) to bind to α -adrenergic receptors in intact platelets⁷. (–)Adrenaline (100 μM) was used to block specific binding sites. Saturation of (–)adrenaline displaceable binding occurred at ~ 2.5 nM ^3H -dihydroergocryptine (Fig. 1). Half saturation occurs at about 1.5 nM. This concentration provides an estimate of the dissociation constant (K_d) of the receptor for ^3H -dihydroergocryptine. There are 0.045 pmol receptors per mg protein.

Citrated platelet-rich plasma was incubated with 100 μM (–)adrenaline or 1 μM phentolamine for up to 6 h at room temperature. Adrenaline did not induce aggregation unless the platelet-rich plasma was stirred and warmed to 37°C. Aliquots of the sample containing (–)adrenaline were stirred in the aggregometer cuvette at intervals to assess aggregation. Control samples which did not contain adrenaline were assessed simultaneously by adding 100 μM (–)adrenaline to an aliquot in the aggregometer cuvette. Binding studies were performed after the aggregation studies.

Figure 2a is representative of 12 experiments illustrating that platelets incubated with adrenaline for 4–5 h become refractory to this hormone as an aggregating agent. After 2 h the time to the initiation of aggregation was prolonged and by 4 h aggregation was no longer observed. The addition of more (–)adrenaline (100 μM) to these platelets also did not cause aggregation. ADP and collagen produced normal aggregation in platelets refractory to adrenaline, suggesting that the desensitisation is specific for the catecholamine. 5-HT release correlated with the aggregation patterns with a decline from 39% to 1% after 4 h of incubation. Figure 2b illustrates that platelet-rich plasma containing no adrenaline still aggregated and released ^{14}C -5-HT, in response to adrenaline, after 5 h of incubation. Desensitisation was also noted when platelet-rich plasma was incubated with lower concentrations of (–)adrenaline (6 μM , 10 μM and 60 μM). In seven experiments at these lower concentrations, (–)adrenaline induced 5-HT release decreased from 39 ± 2.4 to $4 \pm 1.2\%$ ($P < 0.001$) after preincubation with adrenaline for 4 h, while platelets incubated for 4 h in the absence of adrenaline released $33 \pm 1.8\%$ of the ^{14}C -5-HT on addition of the catecholamine.

The status of the α -adrenergic receptor in the incubated platelets was assessed by the ligand binding assay after washing platelets free of plasma or of added catecholamine or phentolamine. In addition to control samples of platelet rich plasma

Fig. 2 Effect of incubation with adrenaline on platelet aggregation and release of ^{14}C -5-HT. Blood anticoagulated with 13 mM sodium citrate was incubated with 1.5 μCi ^{14}C -5-HT for 20 min and centrifuged at 160 g for 10 min at 25°C. The platelet-rich plasma was aspirated and divided into two aliquots. One portion was incubated with 10^{-4}M adrenaline at 25°C. After 0, 2 and 4 h of incubation, 0.4 ml of this platelet-rich plasma was stirred in a Payton dual channel aggregometer at 37°C and the aggregation pattern recorded (Fig. 2a). The percentage of ^{14}C -5-HT released into the plasma following stirring is shown next to each aggregation tracing. The bottom tracing is the aggregation pattern noted when additional adrenaline (10^{-4}M) was added to platelet-rich plasma that had been incubated with adrenaline for 4 h. The second aliquot was incubated for up to 5 h without the addition of adrenaline, and portions were then stirred in the aggregometer immediately after the addition of 10^{-4}M adrenaline (Fig. 2b). After 4 h this platelet-rich plasma aggregated and released ^{14}C -5-HT.



and the samples incubated with 100 μM (–)adrenaline or 1 μM phentolamine for 4 h, 100 μM adrenaline was also added to another aliquot of control platelet-rich plasma just before centrifugation and washing. The duration of this brief exposure to adrenaline was 5 min.

The results of the ^3H -dihydroergocryptine binding studies are shown in Fig. 3. The experimental samples are compared with the control platelets which were not exposed to drugs. The platelets incubated for 4 h with (–)adrenaline showed a decrease of ^3H -dihydroergocryptine binding of $41 \pm 8\%$ ($P < 0.025$), while those incubated with the α -adrenergic antagonist phentolamine did not differ from the control. In contrast, brief exposure of platelets to 100 μM (–)adrenaline increased ^3H -dihydroergocryptine binding $34 \pm 11\%$ ($P < 0.05$).

These data suggest that the loss of aggregation and 5-HT release after exposure of platelets to (–)adrenaline is associated with a decrease in the number of α -adrenergic receptors. In order to prove that (–)adrenaline incubation decreased the number and not affinity of α -adrenergic receptors, a saturation curve was determined for control and (–)adrenaline incubated

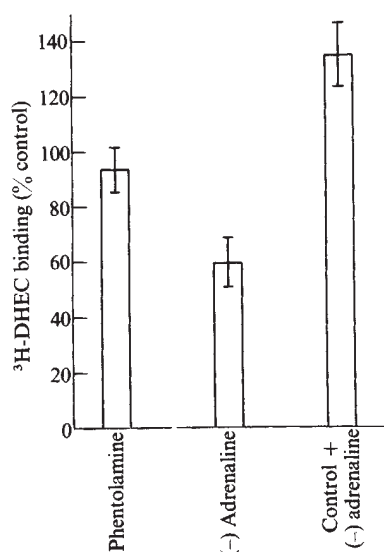


Fig. 3 Regulation of [^3H]-dihydroergocryptine binding by incubation of intact platelets with (–)adrenaline. Platelet-rich plasma was prepared from 250 ml blood anticoagulated with 13 mM sodium citrate by centrifugation at 160 g for 10 min and divided into four aliquots. One portion was incubated with 10^{-4}M (–)adrenaline, a second with 10^{-6}M phentolamine, and a third had no additions (control). After 4 h of incubation when the platelets incubated with (–)adrenaline no longer aggregated in response to this agent, the samples were made 2.5 mM in EDTA and the platelets sedimented by centrifugation at 2,500 g for 5 min. The platelets were then washed twice with 40 ml of buffer containing 8 mM Na_2HPO_4 , 2 mM NaH_2PO_4 , 10 mM EDTA, 5 mM KCl, 135 mM NaCl, pH 7.2. The washed platelet pellet was suspended in the Tris-saline assay buffer at a protein concentration of 3–5 mg ml^{-1} . Just before the washing, 10^{-4}M adrenaline was added to the fourth aliquot of platelet-rich plasma. ^3H -DHEC binding was measured in all four samples in the presence of 2.5–3 nM ^3H -DHEC as described in Fig. 1. The effect of prior exposure to adrenaline or phentolamine on subsequent binding of ^3H -DHEC is expressed as a percentage of the ^3H -DHEC bound by the platelets with no added adrenaline (control). Results are shown as mean $\pm$ s.e.m. for five separate experiments with each point determined in triplicate. Incubation with phentolamine for 4 h did not alter subsequent binding. However, incubation with adrenaline for 4 h decreased binding of ^3H -DHEC by 41% ($P < 0.025$). The addition of 10^{-4}M adrenaline just before centrifugation and washing increased ^3H -DHEC binding 34% (control + adrenaline, $P < 0.05$).

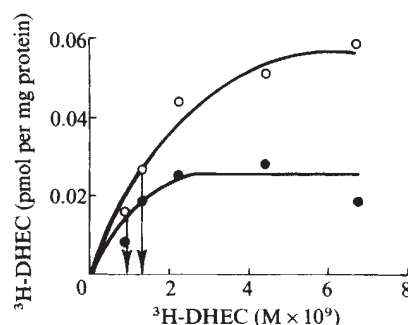


Fig. 4 Saturation of ^3H -DHEC binding sites on control and (–)adrenaline desensitized platelets. Platelet-rich plasma was prepared from 500 ml blood as described in Fig. 3 and divided into two aliquots. One portion was incubated with 10^{-4}M (–)adrenaline (●) and the second with no addition (control, ○) for 4.5 h. At this time the platelets which had been exposed to (–)adrenaline no longer aggregated even with an additional 10^{-4}M (–)adrenaline. The platelets were then prepared for assay as described in Fig. 3 except that the wash buffer did not contain KCl. The assay was run exactly as described in Fig. 1. Each tube contained 0.9 mg protein. The arrows represent half-maximal saturation for each curve.

platelets. In this experiment the preparation of platelets and incubation with 100 μM (–)adrenaline were performed as for Fig. 3. Increasing concentrations of ^3H -dihydroergocryptine were added to a constant amount of control and (–)adrenaline desensitized platelets. As shown in Fig. 4, specific binding for control platelets incubated for 4.5 h in the absence of (–)adrenaline became saturated at ~ 2.5 – 3.0 nM ^3H -dihydroergocryptine with ~ 0.058 pmol binding sites per mg protein. Half saturation occurred at 1.5 nM. These values are quite similar to those derived from Fig. 1 in which the platelets had not been incubated for 4.5 h. In contrast, the platelets incubated in (–)adrenaline had 0.028 pmol of binding sites. The K_d was approximately 1.1 nM. Thus, prolonged incubation of platelets with (–)adrenaline results in a decrease in number but little or no change in affinity of α -adrenergic receptors. This experiment also provides evidence that the washing procedure is adequate since residual (–)adrenaline would be expected to result in an apparent decrease in affinity of ^3H -dihydroergocryptine for the α -adrenergic receptor by shifting the saturation curve to the right at low ligand concentrations. The observation that brief exposure to (–)adrenaline increases ^3H -dihydroergocryptine binding also mitigates against the possibility that residual adrenaline accounts for the changes in binding with prolonged incubation.

The mechanism for, and physiological significance of, the increase in binding following brief exposure to catecholamines are unknown. One possible explanation is that adrenaline induces the appearance of previously unavailable receptors on the platelet surface. An increase in affinity could not explain increased binding at saturating concentrations of ligand.

The mechanism of ligand regulation of the human platelet α -adrenergic receptor is unknown. Strittmatter *et al.*⁵ have shown that the rapid desensitisation of the α -adrenergic receptor of the rat parotid is dependent on membrane voltage and may represent a conformational change. Whether a similar mechanism also explains the much slower time course of desensitisation in the human platelet remains to be determined. However, the time course of desensitisation more closely approximates that of the β -adrenergic receptor of the frog erythrocyte which is not known to be membrane voltage dependent². The fact that an α -adrenergic agonist but not antagonist induces desensitisation and that the exposure to the agonist must be prolonged demonstrates that events subsequent to receptor occupation are necessary. Similar

observations that the ability of ligands to induce receptor desensitisation seems to correlate with their intrinsic activity or ability to activate the receptor have been reported for the β -adrenergic² and insulin¹¹ receptors.

Ligand regulation of receptors may be a general mechanism by which cells modulate their physiological responsiveness¹². The platelet represents an ideal model for studying the regulation of the human α -adrenergic receptor. This system offers the potential for gaining new insights into the physiology of the human α -adrenergic receptor in general and its relevance to platelet physiology and pathophysiology in particular.

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Do presynaptic autoreceptors control dopamine release?

MANY studies have attempted the elucidation of the homeostatic mechanisms controlling the amount of neurotransmitter released in the synapse. There is convincing evidence that the release of noradrenaline (NA), from both peripheral and central presynaptic nerve endings, can be modulated by a negative feedback mechanism, whereby the neurotransmitter present in excess in the synapse depresses its further release by activating specific presynaptic α -receptors. α -Agonist drugs interact similarly to NA with these receptors and inhibit the depolarisation-induced release of the amine, whereas α -antagonists counteract this inhibitory effect^{1–10}. The appealing concept of a feedback control of neurotransmitter release, although unequivocally demonstrated only for NA, has been generalised to other transmitters. This generalisation seems rather premature. In particular, the existence of presynaptic autoreceptors controlling dopamine (DA) release is often accepted^{2,7,11,12}; however, recent observations^{13,14} argue against this interpretation and, as pointed out by Langer¹⁵, the presence of a direct control of DA release by presynaptic receptors remains an open question. In our studies on DA release described here, our experimental approach differs in two main aspects from those used previously^{13,14}. First, we have

monitored the release of ³H-DA synthesised from ³H-tyrosine, in addition to that of exogenous ³H-DA previously taken up by the nerve terminals. Newly synthesised DA is preferentially released by depolarising stimuli¹⁶ and may therefore be preferentially influenced by a feedback mechanism. Second, we have used superfused synaptosomes. In our conditions^{17,18}, the DA released is immediately removed from the nerve endings and any autoinhibition of release is minimised. Therefore, presynaptic receptors are completely available to the agonists (which should decrease the depolarisation-induced release) or the antagonists added to the superfusion fluid. Our results show that the DA agonists have no effect on the stimulus-coupled release of DA, either taken up or newly synthesised, from striatal synaptosomes, and therefore militate against a direct control of DA release through presynaptic autoreceptors similar to that demonstrated for NA.

Synaptosomes were prepared from the corpus striatum of adult male Wistar rats. The crude synaptosomal pellet¹⁹ was resuspended in 0.32 M glucose (at a protein concentration of 6–8 mg ml⁻¹), diluted 1:10 with Krebs–Ringer medium and equilibrated for 10 min at 37 °C in a rotary waterbath. In one set of experiments, the synaptosomes were prelabelled with 0.1 μ M ³H-DA for 10 min and aliquots of the suspension were superfused in several parallel superfusion chambers^{17,18}. Veratridine or high K⁺ were used as depolarising agents, apomorphine or DA as dopaminergic agonists and haloperidol as an antagonist. Details concerning the addition of these substances to the superfusion media are given in the figure legends. In another set of experiments, the equilibrated suspension was distributed among various chambers, superfused with ³H-tyrosine and then with media containing depolarising agents and apomorphine as detailed in the legend to Fig. 2. The media used after the ³H-tyrosine labelling period contained α -methyltyrosine, to stop DA synthesis.

The effect of apomorphine on the release of ³H-DA previously taken up by synaptosomes was tested in a variety of experimental conditions (56 or 24 mM KCl or 10 μ M veratridine, Ca²⁺ concentrations ranging from 2.7 to 0.4 mM, pretreatment with apomorphine or simultaneous addition of the drug with the depolarising agents). In none of these conditions did the DA agonist have a significant inhibitory effect. Figure 1a shows a representative set of experiments. Also, when DA was used as an agonist (in the presence of the DA carrier blocker nomifensine²⁰ to prevent the displacement of ³H-DA by the unlabelled amine¹⁴), no inhibitory effect on the stimulus-coupled release of ³H-DA was observed (Fig. 1b). Haloperidol (Fig. 1c) increased the spontaneous release of ³H-DA, but decreased that elicited by depolarisation (after correction for the effect on spontaneous release). Other authors who studied the effect of neuroleptics on the release of radioactive DA preaccumulated by brain slices or synaptosomes obtained similar results^{13,21,22}. It should be noted that the effect of neuroleptics is the opposite of that expected for a presynaptic antagonist.

To test whether DA agonists act on the efflux of the recently synthesised amine, we had to circumvent the difficulty that DA agonists inhibit synaptosomal DA synthesis^{22,23}, for a decrease in synthesis would result in a decreased release. The decrease in DA release reported by Plotsky *et al.*¹² in brain slices preincubated with apomorphine can be interpreted in this light. Also, in our experimental conditions, superfusion of ³H-tyrosine-treated synaptosomes with apomorphine (1 μ M) led to a decrease in the spontaneous as well as the veratridine-induced release of ³H-DA (unpublished). Superfusion with α -methyltyrosine, an inhibitor of catecholamine synthesis, led to a similar reduction of ³H-DA release. If DA agonists had an effect on release independent of that on synthesis^{7,11}, an inhibition of release should also be detectable when DA synthesis is stopped after labelling with ³H-tyrosine. However, Fig. 2 indicates that ³H-DA release, elicited either by veratridine or by high K⁺, was not inhibited by apomorphine in the presence of α -methyltyrosine.

Fig. 1 Effect of dopamine agonists and antagonists on the depolarisation-induced release of ^3H -dopamine preaccumulated by striatal synaptosomes. Striatal synaptosomes, prelabelled with $0.1\ \mu\text{M}$ ^3H -DA, were superfused on Millipore filters (about $300\ \mu\text{g}$ protein per filter) in a series of parallel superfusion chambers^{17,18}. *a*, During the first minutes, up to the moment of stimulation, the synaptosomes were superfused with standard Krebs-Ringer medium ($2.7\ \text{mM}$ CaCl_2), with or without $1\ \mu\text{M}$ apomorphine. From seven minutes onwards (see arrow) the media contained $10\ \mu\text{M}$ veratridine as a depolarising agent. Incubation and superfusion media contained $12.5\ \mu\text{M}$ nialamide and $1\ \text{mM}$ ascorbic acid. The superfusion rate was $0.5\ \text{ml min}^{-1}$; 1-min fractions were collected and the radioactivity was counted in all the fractions and in the filters at the end of superfusion. The radioactivity present in each fraction was calculated as a percentage of the total radioactivity recovered (fractions plus filter). Each curve is the average of three experiments run in triplicate. *b*, After a period of superfusion with standard medium, the medium was replaced (see arrow) with new media containing $24\ \text{mM}$ KCl (substituting an equimolar amount of NaCl), with or without unlabelled DA and nomifensine as indicated. For other technical details see *a*. Each curve is the average of two experiments run in triplicate. *c*, After a period of superfusion with standard medium, the medium was replaced (see arrow) with media containing veratridine and/or haloperidol as indicated. The increase of ^3H -DA release above control caused by haloperidol alone was subtracted from the release elicited by haloperidol plus veratridine, to evaluate the effect of haloperidol on the release induced by depolarisation (dashed line). The curves presented are the average of three experiments run in triplicate.

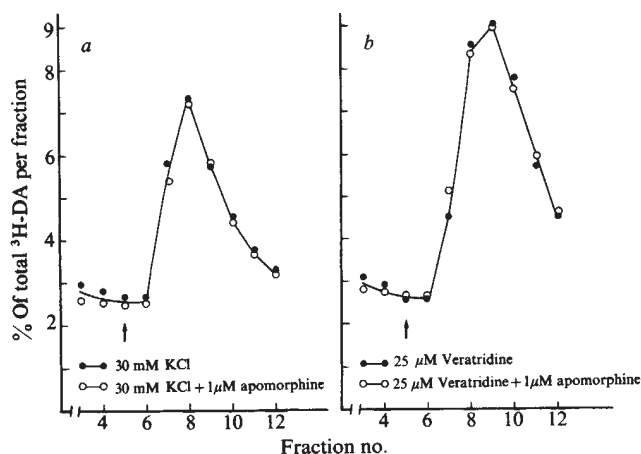
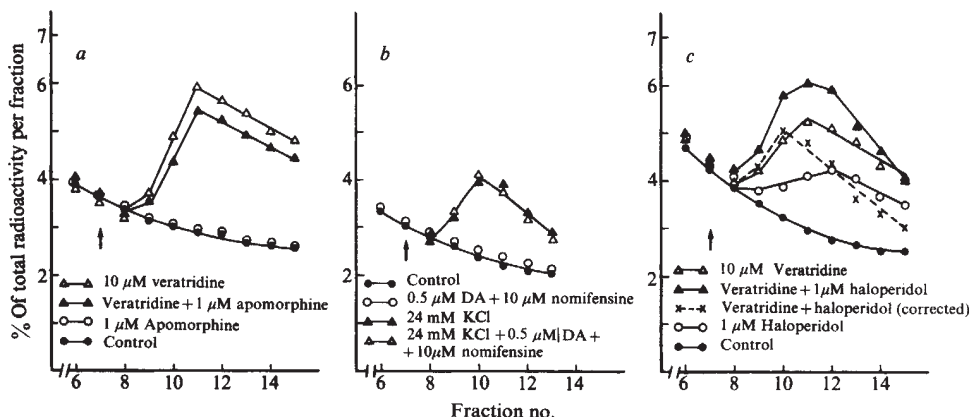


Fig. 2 Effect of apomorphine on the depolarisation-induced release of ^3H -dopamine synthesised from ^3H -tyrosine in striatal synaptosomes. After a 10-min equilibration at 37°C in a rotary waterbath, striatal synaptosomes were plated on Millipore filters (about $700\ \mu\text{g}$ protein per filter) in several parallel superfusion chambers, superfused for 10 min with standard medium containing $0.3\ \mu\text{M}$ ^3H -tyrosine, washed twice with $15\ \text{ml}$ of medium containing $0.1\ \text{mM}$ α -methyltyrosine, under moderate vacuum, and then superfused as follows. *a*, During the first 5 min, synaptosomes were superfused with a medium containing α -methyltyrosine, with or without $1\ \mu\text{M}$ apomorphine. When indicated by the arrow, $30\ \text{mM}$ KCl was added as a depolarising agent. The concentration of CaCl_2 in all the media was $1.2\ \text{mM}$. Fractions were collected every minute into vials containing $100\ \mu\text{l}$ of a protective solution (1.5% EDTA, 1% ascorbic acid and 0.001% unlabelled DA)¹⁶ and the radioactivity released as ^3H -DA was measured in each fraction after isolation of ^3H -DA on Biorex columns²⁴. The radioactivity remaining in the filters at the end of the superfusion was extracted with $1\ \text{M}$ formic acid- $1\ \text{M}$ hydrochloric acid ($85:15\ \text{v/v}$) and the ^3H -DA measured by the same method²⁴. The total radioactivity recovered as ^3H -DA in the effluent plus filter of the controls did not differ from that recovered in the apomorphine-treated samples, indicating that the α -methyltyrosine added after the labelling with ^3H -tyrosine was an effective blocker of the synthesis (see also text). Each curve is the average of three experiments in sextuplicate. *b*, Technical details as in the experiments of *a*, except that $25\ \mu\text{M}$ veratridine was used as a depolarising agent and the concentration of CaCl_2 was $2.7\ \text{mM}$. The curves presented are the average of three experiments in sextuplicate.

In conclusion, our data strongly suggest that neither the release of the DA recaptured through the reuptake process, nor that of the amine newly synthesised from tyrosine is modulated directly through a negative feedback mechanism involving presynaptic receptors. As a control of DA synthesis at the tyrosine hydroxylase level, mediated by the activation of presynaptic receptors, seems well established^{11,22,23,25,26}, the regulation of the transmitter released into the synapse is likely to occur indirectly through this process. The homeostatic mechanism controlling the synaptic concentration of DA would thus differ profoundly from that occurring at noradrenergic synapses, where the release rather than the synthesis of the neurotransmitter is modulated through the presynaptic α -receptors and any effect on synthesis seems to be secondary to that on release. This view is in keeping with the observations that the effects on NA synthesis caused by drugs active on presynaptic α -receptors are abolished in the absence of impulse flow²⁷, whereas in the same conditions the effects of DA agonists and antagonists on DA synthesis remain largely unchanged^{22,23,25}.

It may seem that, whether the mechanism of presynaptic modulation of release is direct (NA) or indirect through synthesis (DA), the ultimate result in terms of neurotransmitter availability in the synapse is the same. However, the existence of one or the other mechanism may be significant. For example, the administration of a catecholamine precursor such as L-dopa, which bypasses the rate-limiting tyrosine hydroxylation step, will lead, in the dopaminergic nerve endings, to a cycle of DA synthesis-release (and reuptake-release) free of any presynaptic control. The lack of a direct control on release may allow the viable dopaminergic nerve terminals of parkinsonism patients treated with L-dopa to compensate, with a higher than normal release of DA, for the decreased number of functioning nerve endings.

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Internal desynchronisation of bilaterally organised circadian oscillators in the visual system of insects

MANY functions of multicellular organisms follow such a consistent daily periodicity, even in constant environmental conditions, that it has been natural to postulate control by a single internal clock. But some observations are explained only by the joint action of several circadian oscillators^{1,2}. Experimental confirmation of such a system has been obtained for several species^{3,4}. For example, two rhythms in an organism may have independent period lengths, even in steady state. This internal desynchronisation is thought to have been demonstrated when the phase difference between the two rhythms exceeds 360° (ref. 5). It has been described for

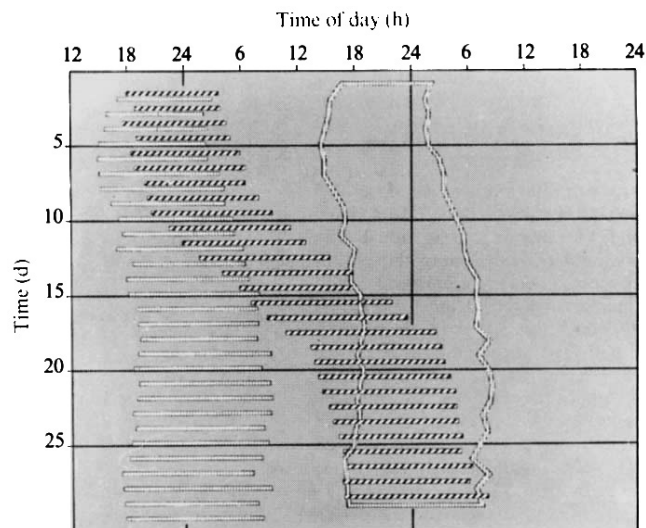


Fig. 2 Independent circadian rhythms of sensitivity in the right and left compound eye of *Blaps gigas* in DD. The horizontal bars represent the circadian night phases in successive days of the experiment. The envelope of the night phases of the left eye is repeated once. Diagonal shading indicates night phase, right eye; vertical shading indicates night phase, left eye.

humans¹ and non-human primates⁶. The possession of pairs of organs by bilaterally symmetrical metazoans suggests an anatomical basis for multioscillator organisation. So far, however, evidence has been based on surgical isolation of the oscillators of molluscs⁷ and crustaceans⁸, or indirectly on ablation experiments with insects^{9–11}. We have now obtained direct evidence with beetles, by demonstrating internal desynchronisation between the right and left sensitivity rhythms in the compound eyes, without surgical intervention.

Fully automated apparatus^{12,13} was used to record the endogenous changes in sensitivity in the compound eyes of tenebrionid beetles of the genus *Blaps*. At intervals of 30 min the eyes were exposed to a 30-ms flash of light of low but constant intensity, and the on-effect amplitude of the electroretinogram (ERG) was recorded (Fig. 1). Such test stimuli elicit ERGs with amplitudes that oscillate in a circadian rhythm in constant conditions. In maintained darkness (DD) the eyes are 10–100 times more sensitive during the circadian night than during the circadian day¹⁴. This phenomenon is caused by a circadian

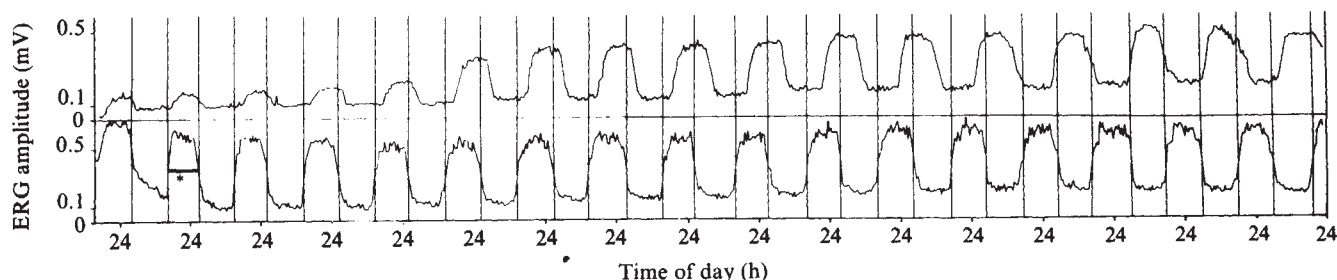


Fig. 1 Right-left-desynchronisation of circadian rhythms of sensitivity in the two compound eyes of *Blaps gigas* during maintained darkness. Beetles were collected on the Island Djerba (Tunisia) *Blaps gigas* L. was used, but *B. requienii* Sol also shows internal desynchronisation as described here. Beetles were waxed to a holder by the back. The recording electrode, inserted into the compound eye, was a 30-μm thick platinum wire; a similar wire implanted in the head capsule at the ventrocaudal edge of the eye served as a reference electrode. At 30-min intervals a brief (30 ms) test stimulus was presented by means of fibre optics and the on-effect amplitude of the ERG was recorded. The upper record shows the right eye; the lower record shows the left eye. Different period lengths cause right and left eye to reach opposite states after 14 d: circadian night in left eye occurs during circadian day in right eye. The vertical lines mark the middle of the transition between day and night phases of the left eye. The horizontal line marked with an asterisk designates a circadian night phase; the times of occurrence of the night phases are plotted in Figs 2–4.

pupillary response similar to that described in the eye of the related beetle *Tenebrio*¹⁵. In DD and constant temperature ($24 \pm 1^\circ\text{C}$) there are interindividual differences in period length (τ); as a rule τ is greater than 24 h.

The beetles had been kept in natural light conditions for several weeks before the experiments. The ERG recorded simultaneously from the two eyes of *Blaps gigas* indicates that in maintained darkness their circadian rhythms remained synchronous at least for some days. But later, in some animals after only a few days, the length of period in the right and left eye began to differ (Figs 1, 2). The difference in phase-angle increased with each cycle, sometimes reaching and even exceeding 360° (compare Figs 3 and 4). Thus the criteria for internal desynchronisation⁵ of the circadian sensitivity rhythms of the two compound eyes are met. Often, however, the beetles died before a phase-angle difference of 360° was attained. For this reason we looked for a way to produce desynchronisation reliably, but without surgery.

When one compound eye of an insect is illuminated there is no light adaptation of the contralateral eye as there is with the consensual pupil response of mammals. Even within a single eye of *Blaps* a stimulus of light causes only local adaptation of the part of the eye illuminated. It was thus possible to use fine fibre optics to subject part of an eye to regimes of constant light

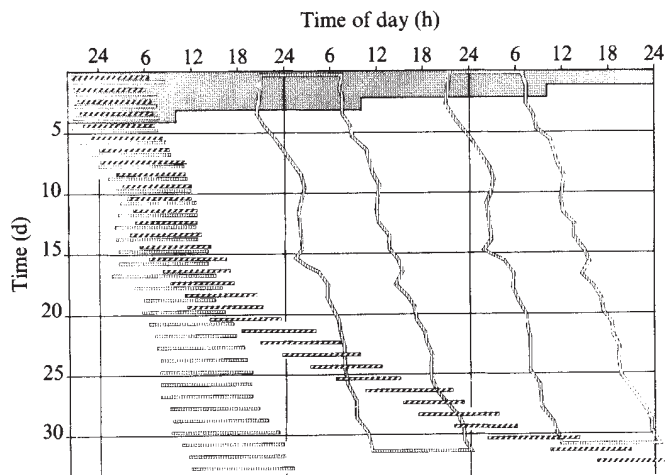


Fig. 3 Sensitivity diagrams plotted as in Fig. 2, except that from the 5th day onwards the right eye was exposed to LL while the left eye remained in DD. The envelope of the night phases of the left eye is repeated twice. After illumination the right eye lengthens its period by more than 3 h. The left eye overtakes the right eye twice during the experiment. Shading as in Fig. 2.

(LL) and light-dark (LD) without superimposing light adaptation on the circadian sensitivity change in another part of the eye or in the contralateral eye.

If part of an eye is exposed to maintained light its circadian period is extended in accordance with Aschoff's rule for night-active animals, while the periodicity of the contralateral eye shows little response. Figure 3 suggests that there was some effect on the contralateral eye, its period being extended by about 0.5 h, but because of the small number of periods in the preceding DD conditions the effect is not established with certainty. The illuminated eye, by contrast, exhibited an extension of average period length by more than 3 h; starting in synchrony with the contralateral eye, it was overtaken by that eye twice during the experiment. As the two rhythms diverged, only the illuminated eye exhibited the large change in period; the other eye fluctuated about the period $\tau = 24.6$ h, which it maintained from the onset of illumination.

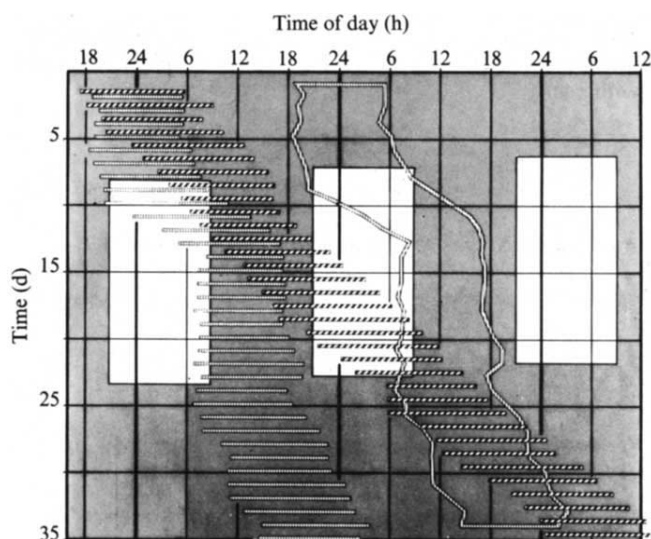


Fig. 4 Sensitivity diagrams plotted as in Fig. 2, except that the left eye was illuminated with an LD timing signal from 9th day to the 23rd day; the right eye was in DD during the entire experiment. The envelope of the night phases of the left eye is repeated once. LD-zeitgeber on left eye fails to entrain right eye. Shading as in Fig. 2.

If one eye was illuminated with a zeitgeber (L:D = 12:12 h) only that eye became entrained to the signal period $T = 24$ h (Fig. 4). The compound eye that remained in DD continued to drift with period unchanged ($\tau = 25.5$ h), not responding with a change in period length even when overtaken by the other eye. In subsequent DD conditions the two rhythms remained desynchronised. When the phase-angle differences are small, synchrony can often be observed for short times (Fig. 4, 24th day). We have been unable to find clear effects of the two rhythms on each other in the amplitude curves of the test ERG (Fig. 3), but a systematic analysis might reveal such interactions at certain phase-angle differences.

Because our results meet the criteria for internal desynchronisation between the circadian rhythms of the two compound eyes, we interpret them as direct evidence of two independent circadian oscillator systems, the output of each controlling light sensitivity in one of the two eyes. The circadian oscillator system of *Blaps gigas*, then, displays a bilaterally symmetrical organisation at this level. This inference seems to be valid for *Tenebrio molitor* as well (G. Schneider, personal communication).

Furthermore, the appearance of internal desynchronisation in such a system calls into question the existence of a superordinate master-clock. Whereas ablation experiments on the visual system of the cockroach¹¹ indicate a bilaterally organised system in which the two oscillators are coupled, in *Blaps* such coupling, if it exists, is very weak. Thus the advantage that bilateral doubling of the circadian system could offer with respect to stabilisation of period length is not utilised at the level we have studied. As seems to be the case in *Aplysia*¹⁶, a compound eye of *Blaps* evidently acts as a zeitgeber receptor only for the ipsilateral oscillator. Synchronisation of the circadian rhythms of the two eyes in the animals' natural surroundings, then, must occur by way of exogenous zeitgebers which of course are available in the daily alternation between light and dark. This finding considerably enlarges the significance of the zeitgeber. In addition to holding the period of the internal clock at the 24-h periodicity of the environment, the zeitgeber provides synchronisation within the multi-oscillator system.

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Mechanism of non-adhesiveness of endothelial and epithelial surfaces

THE luminal surface of undamaged blood vessel endothelium is non-adhesive for platelets, although platelets will adhere to many other surfaces, such as collagen or glass^{1,2}. The mechanism of the non-adhesive nature of the endothelial surface remains unknown. Here we present a hypothesis explaining this non-adhesiveness, based on observations of the behaviour of epithelial sheets in culture. This hypothesis contains three independent suggestions. First, the mechanism of the non-adhesiveness of endothelium is similar to that of the upper surface of cultured epithelial sheets. Second, non-adhesiveness of the upper surface of the epithelium results from an absence of pseudopodial activity. It is suggested that only the surface of pseudopodia extended by the epithelial cells is adhesive, whereas other parts of their surface are non-adhesive. Third, the degrees of adhesiveness of the inactive upper surface and of the pseudopodial surfaces are different as a result of different interactions of membrane components with underlying cortical structures. More specifically, the membrane receptors cross-linked by some ligands from the outside may become 'anchored' at the inner side of the membrane only in the pseudopodia.

Endothelial cells *in vivo* form coherent monolayer sheets at the inner surface of blood vessels³. Endothelial cells in cultures also form monolayers on the substrate^{4,5}. Coherent sheets of similar morphology are formed in cultures by many types of epithelia^{6–11}, and the upper surfaces of various cultured epithelial monolayers are non-adhesive. Pre-labelled homologous epithelial cells, fibroblasts or inert particles (such as carmin or latex) seeded on these surfaces are not attached to them^{7–9,11}. Non-adhesiveness of the luminal surface of endothelia may be regarded as a special instance of the non-adhesiveness of the upper surface of epithelial monolayers.

Microcinematography and scanning electron microscopy of epithelial cultures show that upper surfaces of the cells within

the sheets are nonactive, that is, no pseudopodial protrusions except short microvilli are formed at these surfaces^{6–8,11,12}. Inactivity of these surfaces is probably a result of contact inhibition of movements caused by neighbouring cells in the sheet¹³. This suggestion agrees with the finding that the surface of free lateral edges of epithelial sheets is usually active: pseudopodial extensions (lamellipodia and microspikes) are continually formed along these edges^{7,10,11}. The extended pseudopodia either withdraw or attach themselves to the substrate. A thin cytoplasmic plate ('lamellar cytoplasm') is formed from the attached pseudopodia near the free edge.

In contrast to the upper surface of the central inactive cells, those of the pseudopodia and the lamellar cytoplasm are adhesive. Two epithelial cells spread on the substrate readily form stable lateral contacts through their active edges^{7,8,10,11}. Inert particles are attached to the surface of pseudopodia and the lamellar cytoplasm at the free edges. This attachment is followed by phagocytosis of the particles by the marginal cells of the sheet^{8,11}. These results suggest that cell–cell and cell–particle adhesion in the epithelium is mediated by pseudopodia formed at the free edges of epithelial sheets. A special role of pseudopodia in adhesion was also suggested by the experiments with cells of other types (see refs 1, 12 for reviews). An undamaged endothelial sheet *in vivo* has no free active lateral edges. This may be the cause of the non-adhesiveness of this sheet. It should be noted that the endothelium of the capillaries of certain organs (liver, bone marrow) has a marked ability for phagocytosis. This endothelium may be an exceptional case that needs special investigation. Besides their different adhesiveness, active and non-active parts of the cell surface also differ in their ability to be cleared of membrane receptors cross-linked by external ligands. This was shown in experiments with mouse kidney epithelium treated with concanavalin A or cationised ferritin^{14,15}. Initial distribution of the receptors of these ligands on the surface of epithelial cells was diffuse. Treatment of living cells with one of the ligands induced redistribution of corresponding receptors. In epithelial cells, as in other cell types^{16–20}, redistribution of cross-linked surface receptors had two components: collection of these receptors into microscopically visible groups (patching) and selective removal of the patched receptors from certain parts of the surface (capping or clearing). Patching was observed on all parts of the upper surfaces of epithelial sheets. In contrast, patched receptors were removed only from the pseudopodia and lamellar cytoplasm of the marginal cells of the sheets; clearing was not observed on the upper surface of the inactive central cells of the sheets. Selective clearing of cross-linked surface receptors from pseudopodia was also observed in other cell types^{14,15,17,18,20}.

Patching is usually regarded as a passive aggregation of molecules diffusing in a fluid lipid bilayer. In contrast, capping is an active energy-requiring process. Several theories of the mechanism of this process have been proposed. One plausible suggestion¹⁶ is that capping results from anchoring of the patched receptors to some cortical structures, such as microfilaments; these cortical structures may then actively translocate the patches in the plane of the membrane. Developing this hypothesis, we may assume that anchoring of the patches can take place only in active parts of the cell surface. This suggestion explains not only selective clearing of the patched receptors from these parts of the surface but also their special adhesive properties. Formation of an adhesive structure between the cell membrane and another surface may be somewhat similar to that of patching and capping. The adhesive structure may be regarded as a group of membrane receptors linked from the outside to the molecules of the other surface directly or through some intermediate molecules. This group of receptors is equivalent to a patch formed by an external ligand. Specific ligands and receptors forming different types of adhesive structures have not yet been identified. The 'contact patch' will be unstable in the sense that it may be easily displaced in the plane of the fluid membrane or even detached from this

membrane. This displacement and detachment can be caused by the tension from the adhering cell^{21,22} or by any other mechanical stress acting from the adhering surface. The anchoring of the patched membrane molecules to the underlying cortical structures may stabilise the contact. Alterations of the cortical fibrillar structures observed in the area of specialised cell-cell contacts²³⁻²⁵ may be morphological manifestations of this anchoring. Formation of cell-cell adhesion requires the pseudopodial activities of both adhering surfaces as the receptors have to be anchored in both membranes. Cell particle and cell substrate adhesion require the rigidity of a noncellular surface²² as well as the anchoring of membrane receptors attached to this surface. Nonadhesiveness of inactive epithelial and endothelial sheets may be due to the inability of their surface to attach the membrane receptors adequately to the external ligands.

Other mechanisms preventing platelet attachment to endothelial surfaces, such as production of platelet-affecting substances by the cells of the vessel wall²⁶, may act cooperatively with the mechanism described here.

Our hypothesis provides the basis for designing general experiments. In particular, it would be important to compare adhesiveness of the surface of epithelial sheets for platelets. According to our hypothesis, the surface of artificial fluid lipid membranes is similar to the non-adhesive epithelial surface in one respect: it is unable to withstand tension from the adhering cells. Experiments carried out in this laboratory²⁷ show that fibroblasts cannot attach themselves to the surface of artificial membranes made from the lipids which are in a fluid state at 37°C. Attachment of platelets to such membranes obviously deserves investigation. These experiments are now in progress.

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Isolation, structure and biological activity of two cholecystokinin octapeptides from sheep brain

SEVERAL peptides—for example, substance P and neurotensin—are now known to occur in both endocrine cells of the gut and in central or peripheral neurones¹. The significance of this dual distribution is far from fully understood, but it seems possible that the same peptide might function as both hormone and neurotransmitter. Vanderhaeghen *et al.* have reported that radioimmunoassays (RIA) for the antral hormone gastrin have shown activity in extracts of brain, particularly cerebral cortex, of a wide range of vertebrate species². However, gastrin has the same COOH-terminal pentapeptide sequence as the intestinal hormone cholecystokinin (CCK) and antisera specific for the COOH terminus of gastrin usually cross-react to some extent with CCK also. We found that the pattern of cross-reactivity of boiling water extracts of brain with six different antisera resembled that of a COOH-terminal fragment of CCK more closely than gastrin, and gel filtration studies have indicated that the main component in the extracts was compatible with the COOH-terminal octapeptide of CCK (CCK8)³; these findings have since been confirmed by others⁴⁻⁶. We have purified from sheep brain two octapeptides corresponding to the CCK-like component previously identified by RIA, and report here the isolation, sequence and some properties of these molecules.

Sheep brains were collected from a local abattoir in batches of 5-10 kg and transported to the laboratory where they were immediately boiled in water (70 l) and homogenised. Initial steps of purification were the same as those described for the isolation of big and little gastrins^{7,8}, and involved batchwise adsorption on DEAE cellulose, elution with ammonium carbonate, precipitation at acid pH, isopropanol extraction and fractionation on Sephadex G25. CCK8 RIA showed a major

Fig. 1 Fractionation of aqueous extract of sheep brain on DE cellulose column (1.5 × 10 cm), equilibrated in 0.05 M ammonium carbonate and eluted with gradient to 0.5 M ammonium carbonate. CCK-like activity was estimated by RIA using antiserum (L48) raised in a rabbit immunised against synthetic CCK8 conjugated to bovine serum albumin, with ¹²⁵I-labelled synthetic non-sulphated CCK8. On a molar basis, this antiserum cross-reacts almost equally with CCK8, heptadecapeptide gastrin and intact (33-residue) CCK; standards of CCK8 were used routinely.

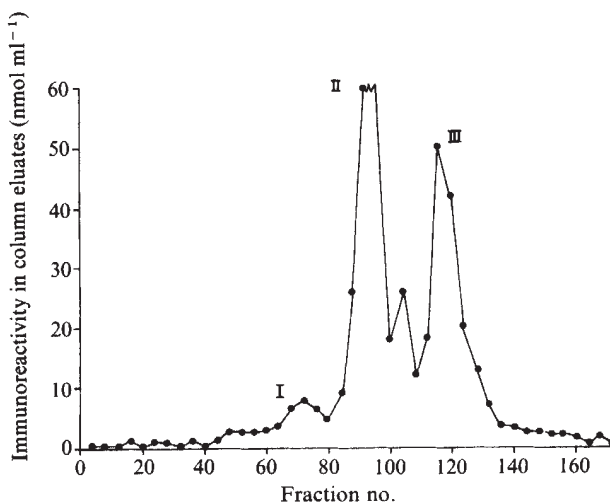


Table 1 Composition of SBII, SBIII and synthetic CCK8

	SBII			SBIII			CCK8		
	a	b	c	a	b	c	a	b	c
Tyr-SO ₃ H		0.7 (1)			0.8 (1)			0.9 (1)	
Aspartic acid*	2.3 (2)	1.8 (2)	1.8 (2)	2.3 (2)	1.9 (2)	1.8 (2)	2.1 (2)	1.9 (2)	2.0 (2)
Glycine	1.3 (1)	1.1 (1)	1.3 (1)	1.1 (1)	1.2 (1)	1.2 (1)	1.3 (1)	1.2 (1)	1.1 (1)
Methionine	1.6 (2)	1.5 (2)	1.5 (2)	1.8 (2)	1.5 (2)	1.7 (2)	1.7 (2)	1.8 (2)	1.9 (2)
Tyrosine	0.8 (1)		1.2 (1)	1.0 (1)		1.3 (1)	0.9 (1)		1.1 (1)
Phenylalanine†	0.8 (1)	1.3 (1)	1.3 (1)	0.9 (1)	1.4 (1)	1.4 (1)	0.9 (1)	1.1 (1)	1.1 (1)
Tryptophan		1.0 (1)	1.0 (1)		1.0 (1)	0.8 (1)		1.1 (1)	0.9 (1)

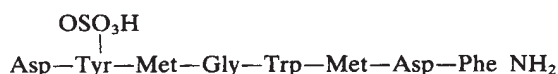
* Identified as NH₂-terminal Asp following dansylation.

† Identified as DNA-Phe NH₂ following 7 cycles of Edman degradation.

a, Analysis following acid (6 M HCl, 105 °C, 24 h) hydrolysis; b, analysis following enzymatic hydrolysis; c, analysis following mild acid (6 M HCl, 105 °C, 2 h) hydrolysis.

peak of activity in the post-salt region of Sephadex G25 column eluates corresponding in elution volume to synthetic CCK8. When fractions containing this material were pooled and fractionated on DE cellulose, one minor and two major immunoreactive components were resolved (Fig. 1); the same three components were also identified in simple boiling water extracts of tissue applied directly to DE columns. The three sheep brain components (designated SBI–III) were subsequently purified separately by column electrophoresis, using a system similar to that described for big gastrin⁸, and by further column chromatography on DE cellulose and Biogel P2. The final yield of SBI was about 50 µg from 100 kg brain, but determination of the NH₂-terminal residue by the dansyl method⁹ revealed several DNS-amino acids, indicating that the material was not yet homogeneous. In contrast, SBII and III were each obtained in yields of 150–300 µg from 100 kg brain, representing an overall recovery of about 5%, and in both cases dansylation revealed NH₂-terminal aspartic acid.

Amino acid analysis after total acid hydrolysis (6 M HCl, 105 °C, 24 h) (Table 1) indicated that the composition of both SBII and III was Asp₂, Gly, Met₂, Phe, Tyr. In addition, total enzymatic hydrolysis with a mixture of aminopeptidase M (2.5 µg) and subtilisin (2.5 µg), on 5 nm of peptide, revealed the presence of tryptophan and indicated that tyrosine was present as *O*-sulphate in both peptides (Table 1). Elucidation of sequence was achieved using the dansyl Edman procedure¹⁰ and showed both peptides to have the structure of the COOH-terminal octapeptide of CCK, that is

**Table 2** Biological and immunochemical potencies of sheep brain CCK octapeptides (II and III) relative to synthetic CCK8

	Bioassay			RIA			
	Gall bladder (guinea pig)	Gall bladder (rabbit)	Pancreas (rat)	1296	2716	L2	L48
SBII	0.82	1.33	0.92	1.08	0.91	1.04	1.21
SBIII	1.16	0.85	0.67	0.86	1.09	1.17	1.55

Bioassays were carried out according to published methods^{11–13}, except that rabbit gall bladders were cut in a longitudinal, rather than a spiral, strip¹². Concentrations of sheep brain peptides and synthetic CCK8 were calculated on the basis of a molar extinction coefficient at 280 nm, 5377. Potency of the sheep brain peptides is expressed relative to CCK8 = 1.00. RIAs with antisera 1296, 2716 and L2 were made using ¹²⁵I-labelled G17 and with L48 using ¹²⁵I-labelled non-sulphated CCK8. Dilution curves of SBII and III were parallel to that for synthetic CCK8, and potency is expressed as ratio of concentrations required for 50% inhibition of binding of label to antibody.

The COOH-terminal residue was identified, after seven cycles of Edman degradations, as DNS-Phe NH₂, which in the two-dimensional chromatography system of Woods and Wang¹¹, runs as a unique spot with an *R_f* 0.41 in 0.5% formic acid and *R_f* 0.71 in benzene:acetic acid (9:1). DNS-Phe NH₂, produced by submitting synthetic CCK8 to the same procedure, was used as standard. The structural basis for the differences between the two components is not yet clear. However, in addition to separation on DE cellulose, the three brain peptides were also resolved by thin layer chromatography in a system of *n*-butanol/dioxan/2 M ammonia (40:10:50) (ref. 12), when SBIII was found to comigrate with synthetic CCK8 (*R_F* 0.53) and ahead of SBII (*R_F* 0.44). Moreover, thin layer electrophoresis at pH 10 also separated SBII and III, although the peptides were not resolved at pH 2.1, 3.5, 6.5 and 8.9. The possibility that SBII was the 3-sulphonyl tyrosine analogue was excluded by showing that both SBII and III, like synthetic CCK8 (ref. 13), were readily and completely desulphated by acid hydrolysis (6 M HCl, 105 °C, 2 h) (Table 1), whereas the synthetic 3-sulphonyl analogue was resistant to hydrolysis (1% conversion in the same conditions).

COOH-terminal fragments of CCK larger than the heptapeptide have full biological activity on gall bladder and pancreas, and may even be more potent than intact CCK¹⁴. We therefore compared the potency of SBII and III with synthetic CCK8 in three CCK bioassays: guinea pig gall bladder *in situ*¹⁵, rabbit gall bladder *in vitro*¹⁶, and rat pancreatic enzyme secretion *in vivo*¹⁷. Both brain peptides were found to be almost equipotent with synthetic CCK8 in stimulating gall bladder contraction and pancreatic enzyme secretion (Table 2). In addition, the immunochemical properties of the brain peptides were compared with CCK8 using four antisera known to differ in specificity for gastrin and CCK; the pattern of cross-reactivity of the brain peptides was virtually indistinguishable from synthetic CCK8 (Table 2). We conclude, therefore, that in sheep brain there is a peptide with the chemical, biological and immunochemical properties of the COOH-terminal octapeptide of porcine CCK. There is also present in approximately equal amount a peptide with closely similar biological and immunochemical properties which is slightly less acidic than CCK8.

In addition to the three peptides described here, other CCK-like components have been identified in brain extracts. For example, there is evidence of a minor component with gel filtration properties compatible with a peptide of 4–6 residues³; and there is also a component emerging in the void volume on Sephadex G25 and G50, and presumably therefore a larger component of more than about 40 residues. CCK has been isolated from pig duodenum in the form of peptides of 33 and 39 residues¹⁸, but immunochemical evidence indicates that CCK8 and a peptide intermediate between CCK8 and the larger forms also occur in significant amounts in pig intestine¹⁹.

Muller *et al.*⁴ have reported substantial amounts of immunoreactivity corresponding to the 33 residue form of

CCK in pig brain. If, as seems likely, CCK8 is produced by selective endopeptidase cleavage of CCK33, one might expect to find equimolar amounts of a corresponding NH₂-terminal fragment. The antiserum used by Muller *et al.* was probably specific for the NH₂-terminal region of CCK (ref. 4) and might be expected to cross-react with an NH₂ terminal fragment. In contrast, the antisera used in our studies are COOH-terminal specific and would not cross-react with such fragments.

The peptides we have isolated account for the greater part of COOH-terminal immunoreactive CCK-like peptides in brain. The characterisation of these octapeptides opens the way for studies of their physiological role in the central nervous system, and in particular an examination of possible neurotransmitter roles. However, the possibility that other CCK-like peptides exert important actions and may even be the major active forms in brain cannot yet be excluded.

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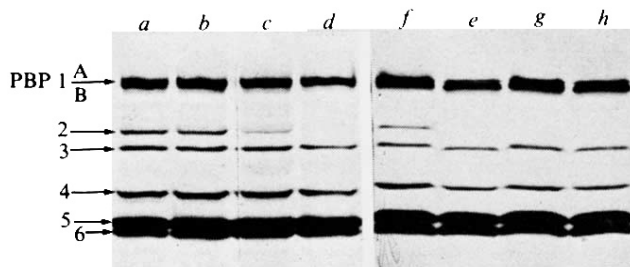


Fig. 1 Thermolability of PBP 2 of mutant SP45 and its derivatives. Cell envelopes were prepared from cells grown in Penassay broth at 26 °C as described¹² except that the buffer used here, and in the PBP assay, was modified to 50 mM sodium phosphate pH 7.0; 10% vol/vol glycerol. ¹⁴C-benzylpenicillin (67 µg per ml final concentration; 58 Ci mol⁻¹) was added to envelopes of KN126 for 20 min at 20 °C (a) or for 10 min at 42 °C after 5 min preincubation at this temperature (b). ¹⁴C-benzylpenicillin (147 µg per ml) was added to envelopes of SP45 for 20 min at 20 °C (c) or for 10 min at 42 °C after 5 min preincubation at this temperature (d). Cell envelopes of SP4504 (e), SP4500 (f), SP4501 (g), and SP4502 (h) were incubated with ¹⁴C-benzylpenicillin (67 µg per ml) for 10 min at 42 °C after 5 min preincubation at this temperature. The binding was terminated and the proteins of the cytoplasmic membrane selectively solubilised and fractionated on a sodium dodecylsulphate polyacrylamide slab gel¹². The PBPs were detected by scintillation autoradiography on Kodak RP Royal X-Omat film¹⁹. PBPs 1A and 1B are not fully resolved on this gel.

bacteria, alteration of the cell envelope resulting in decreased penetration of the antibiotic to the targets responsible for lethality in the cytoplasmic membrane has been proposed^{3,4}. For several groups of antibiotics resistance has been shown to occur by a decrease in the affinity of the target for lethality for the antibiotic^{5–9} but, although this mechanism has been suggested as a possible cause of resistance to β -lactam antibiotics, no examples have been reported. The lethality targets for β -lactam antibiotics in *Escherichia coli* have recently been identified and I report here the characterisation of a mutant that has gained resistance to some β -lactams by a decrease in the affinity of such a target.

Penicillin (and other β -lactam antibiotics) kills bacteria by inhibiting penicillin-sensitive enzymes involved in the final stages of peptidoglycan synthesis^{10,11}. These enzymes are conveniently detected as those proteins of the cytoplasmic membrane that covalently bind ¹⁴C-benzylpenicillin and, when studied in this way, are called penicillin-binding proteins (PBPs)^{10–12}. Seven PBPs have been detected in *E. coli*, and genetic and physiological studies have identified PBPs 1B, 2 and 3 as the targets with which β -lactam antibiotics interact to produce their lethal effects^{11,13–16}. Most β -lactam antibiotics kill *E. coli* by binding to two or all three of these targets and therefore, a mutation that results in a reduction in the affinity of one of the targets for antibiotic will have little effect on the level of resistance of the bacteria as killing will still occur by binding to the other targets. A few β -lactams show exclusive or preferential binding to a single lethality target and in these cases resistance of the organism should occur by a decrease in the affinity of the target for the antibiotic. For example, the amidinopenicillanic acid, mecillinam, binds exclusively to PBP 2 over a very wide concentration range and the inactivation of this PBP by the antibiotic results in the growth of *E. coli* as large spherical forms and consequent cell death^{12,13,17}. As killing by mecillinam is believed to be solely due to the inactivation of PBP 2, it should be possible to find mutants that are resistant to mecillinam because they produce an altered PBP 2 with a decreased affinity for the antibiotic. This type of resistance requires a subtle alteration of the structure of PBP 2 so that it discriminates between the binding of mecillinam and

Escherichia coli resistance to β -lactam antibiotics through a decrease in the affinity of a target for lethality

CLINICAL isolates of bacteria that have gained resistance to β -lactam antibiotics (penicillins, cephalosporins and related compounds) often arise by the acquisition of a plasmid that produces a β -lactamase^{1,2}. An increase in β -lactamase activity has also been shown to cause resistance in some mutants isolated in the laboratory³. In other cases, β -lactamase activity is not the cause of resistance and, at least in Gram-negative

Table 1 Minimal inhibitory concentration ($\mu\text{g ml}^{-1}$) of several β -lactam antibiotics

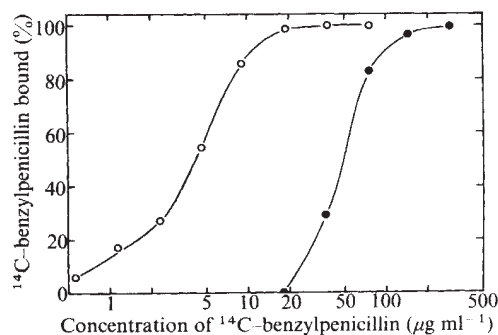
Strain	Mecillinam	Morpholino-amidinopenicillanic acid	Thienamycin	Clavulanic acid	Benzylpenicillin	Cephaloridine
KN126	0.04	0.8	0.6	16	12	1.6
SP45	0.6	6.0	10	64	12	1.6
SP4504	0.04	0.8	0.6	16	25	1.6
SP4500	1.2	12	10	128	25	1.6

Tubes containing 1 ml of Penassay broth and twofold serial dilutions of antibiotic were inoculated with 0.1 ml of a 10^{-4} dilution of an overnight culture of bacteria. After incubation for 32 h at 26 °C the minimal inhibitory concentration was read as the lowest concentration that inhibited turbid growth of the bacteria.

of the normal substrate (β -lactam antibiotics are believed to act as analogues of a part of the substrate of penicillin-sensitive enzymes). In many cases the alteration of PBP 2 is likely to reduce its thermostability and this can be used to recognise mutants that are mecillinam resistant due to a change in PBP 2 from those that are resistant for other reasons; some of the former class will grow as normal rod-shaped cells at 30 °C (where the altered PBP 2 is active) and will be temperature-sensitive and grow into large spherical cells at 42 °C (where the altered PBP 2 is denatured).

E. coli KN126 was plated at 30 °C on Penassay agar (Difco) containing 0.1 μg of mecillinam per ml (twice the minimal inhibitory concentration) and 1 in 2×10^8 cells formed colonies. Two out of 35 of these resistant mutants were rod shaped at 30 °C or below, and grew into large spherical cells at 42 °C. One of them, SP45, was chosen for further study. It grew in minimal and nutrient media at 30 °C with the same growth rate as the parent and was 15-fold more resistant to mecillinam (Table 1). As expected, SP45 had a highly thermolabile PBP 2. No penicillin-binding activity of PBP 2 could be detected in cell envelopes of the mutant with our normal methods¹² but, by using a more protective buffer to prevent the possible inactivation of a highly thermolabile PBP (see legend to Fig. 1), it was possible to detect a low level of activity of PBP 2 at 20 °C (or 30 °C) in cell envelopes prepared from cells grown at the permissive temperature (Fig. 1c). The activity of PBP 2 of the mutant was rapidly eliminated during incubation of the cell envelopes at 42 °C, whereas the activities of all the PBPs of the parent strain were stable in these conditions (Fig. 1).

Fig. 2 The affinity of PBP 2 of *E. coli* KN126 and mutant SP45 for ^{14}C -benzylpenicillin. Cell envelopes of KN126 and SP45 were prepared from cells grown in penassay broth at 26 °C. Twofold increasing concentrations of ^{14}C -benzylpenicillin were added to aliquots of cell envelopes (in 50 mM sodium phosphate buffer pH 7.0; 10% vol/vol glycerol) for 10 min at 30 °C. The binding was terminated and the PBPs fractionated and detected by scintillation autoradiography as described in the legend to Fig. 1. The level of ^{14}C -benzylpenicillin bound to PBP 2 at each penicillin concentration was quantitated by microdensitometry of the X-ray film. ○, KN126; ●, SP45.



Mutants with defects in PBP 2 have been mapped very close to *lip* at about 14 min on the *E. coli* chromosome^{14,16}. The defect in SP45 also mapped at this position, as 84% of *lip*⁺ transductants of *E. coli* AB1325*lip*-9 were temperature-sensitive and grew as spherical cells at 42 °C. Three temperature-sensitive *lip*⁺ transductants (SP4500, SP4501 and SP4502) and one normal *lip*⁺ transductant (SP4504) were shown to have thermolabile and a thermostable PBP 2, respectively (Fig. 1e-h). The temperature-sensitive transductant, SP4500, was further examined and shown to have become resistant to mecillinam when compared to the homogeneous normal transductant, SP4504 (Table 1). The joint transduction of the resistance to mecillinam, the temperature sensitivity of cell shape and the thermolability of PBP 2 imply that these three aspects of the phenotype of SP45 were caused by a single mutation which was presumably in the structural gene for PBP 2. The presence of more than a single mutation in SP45 was also unlikely *a priori*, as the mutant was isolated without mutagenesis.

Two pieces of evidence strongly suggested that PBP 2 of mutant SP45 had a decreased affinity for most (or perhaps all) β -lactams at the permissive temperature. First, direct measurement of the affinity of the mutant PBP 2 for ^{14}C -benzylpenicillin showed that 50% saturation required 44 μg benzylpenicillin per ml for 10 min at 30 °C, compared to 3.9 μg per ml for PBP 2 of the parent strain in the same conditions (Fig. 2). Direct measurement of the affinity of PBP 2 for mecillinam was not possible as radioactive mecillinam was unavailable. Second, it was argued that if PBP 2 of the mutant had a decreased affinity for most β -lactams at the permissive temperature, it should result in cross-resistance to those β -lactams that, like mecillinam, kill *E. coli* at their lowest effective concentrations by inactivation of PBP 2 (for example, the morpholino analogue of mecillinam¹⁷, thienamycin¹⁸ and clavulanic acid¹⁸), but not to the typical penicillins and cephalosporins that kill by inactivation of PBP 1B and/or PBP 3 (refs 11, 13-16) (for example, benzylpenicillin and cephaloridine). Table 1 shows that SP45 and the transductant SP4500 had the expected pattern of cross-resistance to β -lactam antibiotics.

The available evidence suggests that mutant SP45 is resistant to mecillinam at 30 °C or below because it produces an altered PBP 2 with a decreased affinity for mecillinam. At higher temperatures the altered PBP 2 is denatured, resulting in temperature sensitivity of cell growth. The clinical significance of this type of resistance to β -lactam antibiotics is unknown. Some examples of low level resistance of clinical isolates, where β -lactamase activity is absent, could be due to alteration of a lethality target, although this can only occur when the β -lactam kills the organism over a substantial concentration range by inactivation of a single target. High levels of resistance by this mechanism are probably rare as it would depend on the virtual exclusion of the β -lactam from the active site of the lethality target without impairing its thermostability or the binding of the normal substrate.

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IncP2 group of *Pseudomonas*, a class of uniquely large plasmids

In *Pseudomonas* bacteria, the plasmids of the P-2 incompatibility group (IncP2) are notable for several reasons: they include both antibiotic resistance plasmids of nosocomial origin (R plasmids) and degradative plasmids that allow dissimilatory oxidative metabolism of unusual carbon sources; they are a naturally occurring high frequency transfer system with a host range restricted primarily to *Pseudomonas*; they are reported to be the most frequent incompatibility group of R plasmids found in nosocomial *Pseudomonas aeruginosa* isolates; and they show a wide geographical distribution^{1–4}. Until now, their classification as plasmids has primarily been on the basis of genetic evidence: cotransfer of plasmid phenotypic markers independent of chromosome transfer; and co-curing of plasmid markers by another IncP2 plasmid. We have developed a novel method to permit routine physical isolation of these plasmids⁵. Using this method, a survey of the sizes of 12 different IncP2 plasmids reveals them to be notable in another way: they constitute a unique group of very large bacterial plasmids, generally having a homogeneous, unprecedented size range between approximately 280 and 312 megadaltons.

Our plasmid isolation method (summarised in Fig. 1) was developed from the hypothesis of Kline *et al.*⁶ that quantitative nonintegrative attachment of stringently replicating plasmids to the bacterial folded chromosome acts as both the site and the mechanism of replication and segregation of the plasmid⁶. We therefore reasoned that the problem in isolating large plasmids was twofold: they must be separated from the folded chromosome and the procedures must be gentle to avoid hydrodynamic shearing. The inclusion of heat pulses, high sodium dodecyl sulphate concentration, and alkali denaturation in a gentle lysis procedure, permitted isolation of large plasmid DNA and its visualisation by agarose gel electrophoresis. Fortunately, the denaturation step also reduces the amount of broken chromosomal DNA as seen in agarose gels⁵. Plasmids that are not covalently-closed, circular DNA, or plasmids that have alkali-sensitive relaxation complexes would not be recovered by our procedure.

In Fig. 1, two IncP2 plasmids, pMG1 and pMG5, were co-electrophoresed on a single agarose slab gel with four other smaller plasmids. The sizes of pMG1 and pMG5 listed in Table

2 were measured by electron microscopy of satellite DNA taken from an ethidium bromide–caesium chloride density equilibrium gradient. Agarose gel electrophoresis of that satellite DNA gave bands that comigrated with plasmid DNA prepared by our lysis method; thus, the IncP2 plasmid bands seen on agarose gel electrophoresis are considered to be supercoiled, covalently closed circular DNA⁷. In Fig. 1, the plasmid bands have migrated in accordance to size: the smallest plasmid, the LT2 cryptic (60 megadaltons) is furthest from the origin (well *a*), and the largest plasmid, pMG1 (312 megadaltons) has migrated the least distance (well *f*). A standard curve of the six plasmids in Fig. 1 constructed in the method of Meyers *et al.*⁷ (log relative mobility against log molecular weight) does not appear linear for plasmids above about 140 megadaltons⁵. But we considered that we could use pMG5 and pMG1 as relative size standards in agarose slab gel electrophoresis to test whether the IncP2 plasmids were all of a similar size.

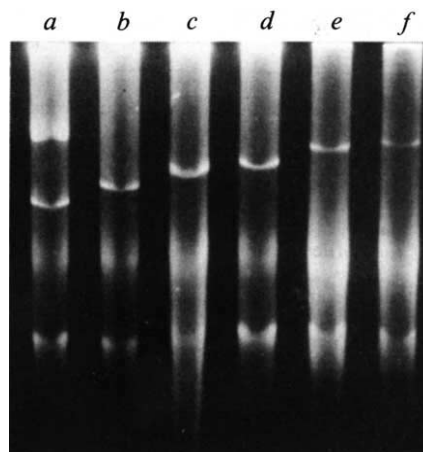


Fig. 1 Preparation of DNA for agarose gel electrophoresis is described in detail in ref. 5. Cells were grown in 40 ml complex broth medium to late log phase, washed and resuspended at high density in buffered 25% sucrose (pH 8.0). All subsequent mixing was done by slow, gentle inversion. To lyse cells, we added lysozyme and ethylenediaminetetraacetate, and then sodium dodecyl sulphate (SDS) to 4% final concentration. Eight repeated cycles of heat pulse and mixing produced a clear, viscous lysate. DNA was denatured at pH 12.1–12.3 by adding 3 M NaOH and mixing for 3 min at room temperature. Then, tris(hydroxymethyl)aminomethane (pH 7.0) was added to return the pH below 9.0. We added SDS to 4% final concentration, NaCl to 1.0 M, and mixed by 20 inversions; after 6 h at 4°C, the salt-precipitated chromosome–membrane complexes were pelleted by centrifugation at 17,000g (4°C, 30 min). The supernatant was mixed with polyethylene glycol 6000 to 10% concentration. After 6 h at 4°C, the tubes were centrifuged at 700g (4°C, 5 min). Resuspension of the resulting pellets in 0.15 ml cold buffer gave 'plasmid-enriched DNA solution'. Agarose slab gel electrophoresis, for 3 h at 100 V through 0.7% agarose (w/v), was carried out using the method of Meyers *et al.*⁷. Each well contained 25 µl of 'plasmid-enriched DNA solution' mixed with 10 µl Meyers tracking dye⁷. Gels were stained with ethidium bromide solution and visualised on an ultraviolet transilluminator. The distinct bands seen are plasmid DNA. The species of the bacterial strains used as input for DNA preparation are indicated by the beginning letters of the strain designation: AA or LT are *Salmonella typhimurium*, DT is *Escherichia coli*, and PAO is *P. aeruginosa*. Input strains for the different wells were: *a*, LT2; *b*, AA2103(F'80his-gnd); *c*, DT41(R27); *d*, DT78(TP116); *e*, PA038(pMG5); *f*, PA038(pMG1). LT2 contains the cryptic plasmid. DNA migration was from top to bottom. The photograph is a single slab gel, with multiple wells; thus, relative size comparisons can be made within the gel.

Table 1 Selected plasmids of the P-2 incompatibility group

Plasmid	Relevant phenotype*	Species that originally harboured plasmid†	Geographical location and source of original plasmid-containing strain (date of earliest report‡)	Ref.
pMG1§	Tra ⁺ TerHgSmSuGmUv	<i>P. aeruginosa</i>	Capetown, South Africa; burn unit clinical isolate; 1974	12¶
pMG2§	Tra ⁺ TerHgSmSuGmUv	<i>P. aeruginosa</i>	Atlanta, Georgia; burn unit clinical isolate; 1974	12
pMG5§	Tra ⁺ TerHgSmPmaKmTmAkBt	<i>P. aeruginosa</i>	Japan; clinical isolate; 1972	12
pMG6§	Tra ⁺ TerHgSmSuCmGmKmTm	<i>P. aeruginosa</i>	Boston, Massachusetts; clinical isolate; 1978	13
Rms159§	Tra ⁺ TerHgSmTcPmaCm	<i>P. aeruginosa</i>	Japan; 1976	12
R38§	Tra ⁺ TerHgSmSuTcPma	<i>P. aeruginosa</i>	Matsumoto, Japan; urinary tract infection clinical isolate; 1972	12
R931**	Tra ⁺ TerHgSmTcUv	<i>P. aeruginosa</i>	Alberta, Canada; clinical isolate; 1972	12
R3108**	Tra ⁺ TerHgSmSuTcPma	<i>P. aeruginosa</i>	Alberta, Canada; clinical isolate; 1973	12
RPL11	Tra ⁺ TerHgSmSuTcPmaCmGmCb	<i>P. aeruginosa</i>	Cincinnati, Ohio; urinary tract infection clinical isolate; 1975	12
RPL11-JH4	RPL11 derivative sensitive to Gm, Tc			This lab.
Cam**	Tra ⁺ TerUvCam	<i>P. putida</i>	Reported from Urbana, Illinois; 1959	12
OCT**	Tra ⁻ Alk	<i>P. putida</i>	Ann Arbor, Michigan††; soil; 1963	12

* Phenotypes for fertility inhibition towards plasmids RP1 and FP2, and phage-growth inhibition of various *Pseudomonas* phages, are not included, but see ref. 12 for a partial list. The Ter phenotype comes from ref. 14; other phenotypes are from ref. 12. Phenotypic designations are as follows: mediating conjugation, Tra; biodegradation of camphor, Cam; biodegradation of alkanes, Alk; and resistances to telluri(a)te, Ter; mercuric ion, Hg; streptomycin, Sm; sulphonamide, Su; tetracycline, Tc; phenyl mercuric acetate, Pma; chloramphenicol, Cm; gentamicin, Gm; ultraviolet light, Uv; kanamycin, Km; carbenicillin, Cb; tobramycin, Tm; amikacin, Ak; butirosin, Bt.

† None of the plasmids used in this work were contained in their original hosts.

‡ The date of the earliest report of the strains from which the various IncP2 plasmids originated necessarily lags behind the actual date of isolation.

§ Plasmids obtained from G. A. Jacoby, Massachusetts General Hospital.

¶ This reference is a review compilation that includes citations of original research papers.

** Plasmids obtained from J. A. Shapiro, University of Chicago.

|| Plasmid obtained from J. C. Loper, University of Cincinnati.

†† Personal communication, M. J. Coon, University of Michigan, Ann Arbor, Michigan.

The comparison of 12 different IncP2 plasmids on two agarose slab gels is seen in Fig. 2. Both gel 1 and gel 2 contain pMG5 and pMG1 as size standards (wells 1b, 1i, 2e and 2f). When PA038 does not contain either pMG5 or pMG1, the plasmid band is absent (well 1a). A similar control with a plasmid-free PU21 is seen in well 2g. Plasmid-free controls of PF0141 and PA02 also show no bands (unpublished data). The *P. putida* strains, PpS731, Pp1142 and Pp1169, are isogenic for chromosome, but differ in the IncP2 plasmids that they contain: variations in plasmid band mobility correlated with the presence of different plasmids. Thus, the bright bands seen in the two slab gels in Fig. 2 are IncP2 plasmid DNA (the lower diffuse fluorescence is the region of chromosomal pieces). In Fig. 2, gel 1, we see that the plasmids R931, R3108, RPL11 and RPL11-JH4 all migrated about as far as pMG5, whereas

the Cam plasmid seemed to be closer in size to pMG1. In gel 2, we see that the IncP2 plasmids pMG2, pMG6, R38 and Rms159 all seem to migrate between pMG5 and pMG1. Measurement of relative mobilities in repeated gels confirms this visual inspection. Therefore, it seems that pMG1 and pMG5 conveniently define approximate upper and lower bounds for at least nine other IncP2 plasmids.

The exception to this was the OCT plasmid (Fig. 2, well 1f) which migrated faster than pMG5. Estimates of OCT's size by comparison on gels with the standards in Table 2 were subject to error, as the standard curve was non-linear and we do not have size standards between 143 and 280 megadaltons. But OCT is larger (migrates more slowly) than TP116 (unpublished data) and its probable size is between 180 and 230 megadaltons. OCT's smaller size compared with other IncP2 plasmids correlates with its transfer deficient (Tra⁻) phenotype (see Table 1). There is evidence that the original parental OCT may have dissociated into three plasmids, OCT, MER and K (ref. 8). The K plasmid is Tra⁺ and will mobilise the transfer of OCT; estimates of its size vary from 65–85 megadaltons^{5,8,9}. Interestingly, if K and OCT were combined, that congregate would fit the size range of the other IncP2 plasmids we have observed. Fennewald *et al.*¹⁰ also have evidence for the large size of IncP2 plasmids. They were not able to isolate unbroken plasmid DNA, but used restriction digests of the linear pieces of plasmid DNA obtained to establish a minimum size estimate of 200 megadaltons for several IncP2 plasmids.

There are reports of plasmids of smaller size isolated from strains that include various IncP2 plasmids, such as R931, Cam, OCT and pMG1 (refs 1, 5, 9). If smaller plasmids are present in our lysates, they must remain a minor proportion, as they are not evident in agarose gels (Figs 1 and 2). We feel that the large plasmids discussed here are the predominant and

Table 2 Plasmids used as size standards in agarose gel electrophoresis

Plasmid	Incompatibility group	Molecular size (megadaltons)	Refs
LT2 cryptic*	Unknown	60	15
F ^{80his-gnd} *	F _I	79	15
R27†	H1	112	16
TP116†	H2	143	16
pMG5‡	P2	280 ± 15	5
pMG1‡	P2	312 ± 17	5

* Plasmids obtained from H. J. Whitfield, University of Michigan Medical School.

† Plasmids obtained from D. E. Taylor, Hospital for Sick Children, Toronto, Ontario, Canada.

‡ See Table 1.

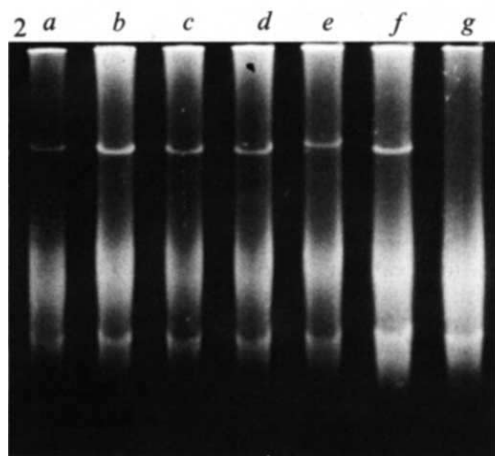
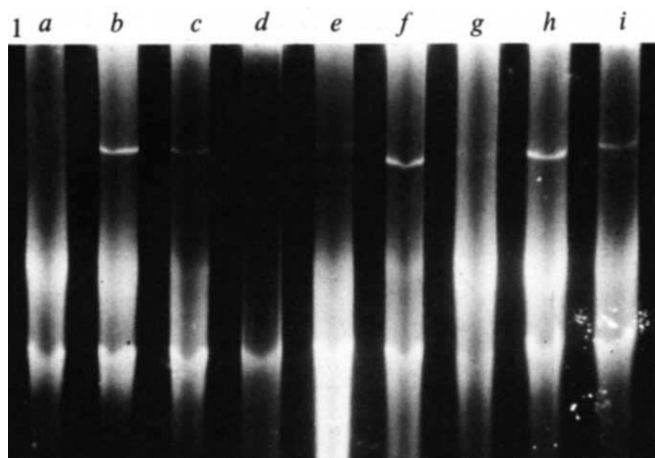


Fig. 2 Each well was loaded with 25 μ l 'plasmid-enriched DNA solution' and 10 μ l tracking dye. For preparation of DNA and electrophoresis conditions, see Fig. 1. The species of bacterial strains used as input for DNA preparation are indicated by the beginning letters of the strain designations: PA0 and PU are *P. aeruginosa*, PFO is *P. fluorescens*, and PpS is *P. putida*. Input strains for the different wells were: 1a, PA038; 1b, PA038(pMG5); 1c, PpS731 (contains R931 plasmid); 1d, PpS733 (contains R3108 plasmid); 1e, PpS1142 (contains Cam plasmid); 1f, PpS1169 (contains OCT plasmid); 1g, PF0141(RPL11); 1h, PA02(RPL11-JH4); 1i, PA038(pMG1); 2a, PU21(pMG2); 2b, PU21(pMG6); 2c, PU21(R38); 2d, PU21(Rms159); 2e, PA038(pMG1); 2f, PA038(pMG5); and 2g, PU21. PA038 and PU21 do not contain any IncP2 plasmids. Each photograph is a single slab gel, with multiple wells; thus, relative size comparisons can be made within each gel.

usual form. But it may well be that IncP2 plasmids can transiently produce smaller derivatives through occasional dissociation.

Inspection of Table 1 demonstrates the geographical diversity (spanning three continents) in origins of IncP2 plasmids. Notably, the two degradative plasmids, OCT and Cam, differ from the IncP2 R plasmids in two ways: they originated in a different species, a saprophytic soil pseudomonad, *P. putida*, and reports of their isolations predates those of the R plasmids by 10 years.

The molecular weight of the *P. aeruginosa* chromosome has been estimated at 2.1×10^9 (ref. 11); thus the IncP2 plasmids are 10–15% of the magnitude of their host's genome. Several questions exist about this prototypical class of very large plasmid. Does not the comparison of their size with their known phenotypes suggest that the plasmids carry many other genes

(perhaps 300 or more) the function of which is as yet unknown? Why is size so widely conserved among the IncP2 plasmids? What natural advantage accounts for their frequent appearance in nosocomial isolates? What is the relationship between IncP2 plasmids from soil isolates and those from the hospital? How much homology exists among the IncP2 plasmids?

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Chloroplast DNA codes for tRNA from cytoplasmic polyribosomes

CHLOROPLAST DNA (ctDNA) from *Euglena gracilis* contains 25–27 cistrons for tRNA^{1,2} and two cistrons each for 16S and 23S tRNA³, and 2.8% of the DNA codes for non-ribosomal RNA containing polyadenosine⁴. The tRNA from purified chloroplasts complements 18 cistrons, whereas whole cells contain species of tRNA complementing 7–9 additional cistrons¹. Chromatography on DBAE-cellulose separates the tRNA into two fractions¹. Uniquely modified bases are found in the fraction II tRNA. Isolated chloroplasts do not contain fraction II tRNA but this tRNA does hybridise to 7 or 8 cistrons on the ctDNA¹. The present results demonstrate that fraction I and fraction II tRNAs are coded by separate and distinct cistrons and that cytoplasmic polyribosomes contain the fraction II tRNA. Further, the aminoacyl acceptor species in fraction II tRNA from whole cells and from cytoplasmic polyribosomes are the same, but the relative abundance of each species varies in the two samples.

A saturating level of total cellular tRNA was hybridised to ctDNA and competed with total cellular tRNA, 16S and 23S rRNA, or fraction I tRNA, (Fig. 1). All hybridisation reactions saturated the ctDNA within a precision of $\pm 10\%$ in 4–20 repetitions of the experiments. Typical results of competition reactions are shown in Fig. 1. The zero point, 100%, is equal to 25 cistrons. The total cellular tRNA competes to the theoretical level (91%, for a 10-fold excess of competing RNA) and validates the hybridisation reactions. The 16S and 23S rRNA (present in equivalent amounts) did not affect the hybridisation

and shows that the DBAE-cellulose effectively removes degraded rRNA which sometimes contaminates tRNA preparations from *Euglena*². Fraction I tRNA competed with 59% of the total cellular tRNA. Subtracting the 9% level of the theoretical non-competed from the remaining 41% leaves 32% of the 25 cistrons—that is, 8 cistrons not competed. This latter number of cistrons is the same as that indicated by hybridisation of Fraction II tRNA. In summary, fraction I and fraction II tRNAs complement separate and unique cistrons.

Cytoplasmic polyribosomes, whose rRNA is coded by nuclear DNA, were analysed in an attempt to identify the subcellular location of the fraction II tRNA. Cytoplasmic polyribosomes were isolated and identified as described previously⁶. No detectable levels of chloroplast subunits, ribosomes or polyribosomes were found in these preparations. Further, only cytoplasmic rRNA was identified from these polyribosomes following phenol extraction and electrophoresis in polyacrylamide gels. tRNA was isolated from the cytoplasmic polyribosomes and separated into fraction I and fraction II as before. Fraction II was confirmed as tRNA by electrophoresis in polyacrylamide gels and aminoacylation as described previously¹. That fraction II tRNA from cytoplasmic polyribosomes is coded by ctDNA was confirmed by hybridisation experiments. Figure 2 is a typical experiment and shows that 0.22% of the ctDNA, or 8 cistrons, hybridised at saturation. This equals the extent of hybridisation reported¹ for fraction II tRNA from whole cells. Thermal denaturation indicates that the binding of tRNA to ctDNA represents specific hybrids. Denaturation occurs sharply between 55 and 90 °C with a T_m of 73 °C in standard saline citrate (Fig. 3).

Fig. 1 Total cellular tRNA hybridisation to ctDNA with rRNA, fraction I tRNA and total cellular tRNA as competitors. Total cellular tRNA, fraction I tRNA and ctDNA were prepared as described previously¹. 16S and 23S rRNA were prepared as described by Mielenz *et al.*³. The total cellular tRNA was labelled *in vitro* with ¹²⁵I and hybridised to ctDNA as reported previously¹. Each reaction mixture contained a saturating level of radioactively labelled total cellular tRNA (1,000 ng with a specific activity of 1×10^7 c.p.m. per μ g tRNA) and 163 ng ctDNA on each filter. *Escherichia coli* DNA was used as a heterologous control. The level of hybridisation was at least five times above the background radioactivity bound to the *E. coli* DNA filters which was 2×10^{-4} of the level of the total radioactivity in each reaction mixture. Increasing amounts of nonradioactive RNAs were added to the reactions. ●, 16S and 23S rRNA; □, total cellular tRNA; ■, fraction I tRNA as the competing species. Total cellular tRNA competes to the theoretical level of 91% at a 10-fold excess of nonradioactive tRNA. Fraction I tRNA competes with all but 41% of the total tRNA hybrids. The rRNA has no effect on the tRNA hybrids.

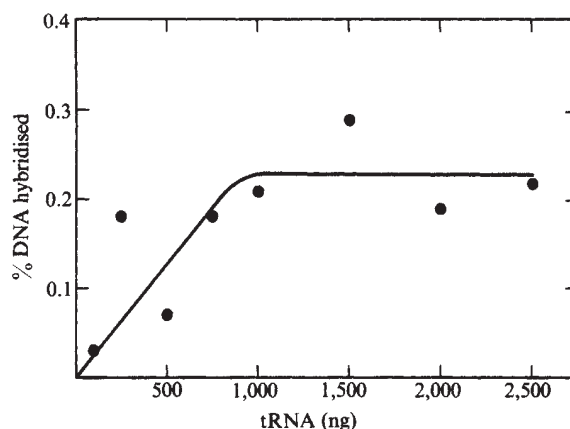
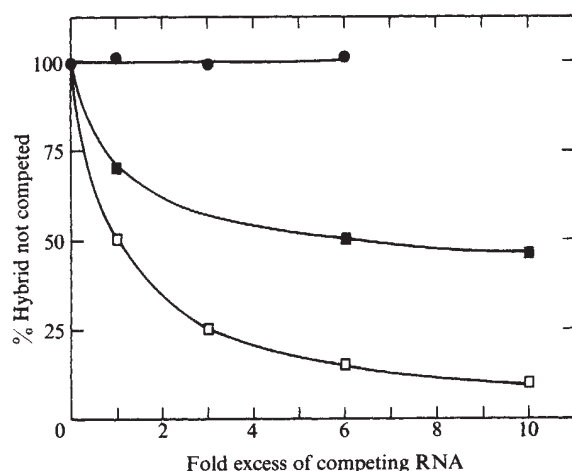
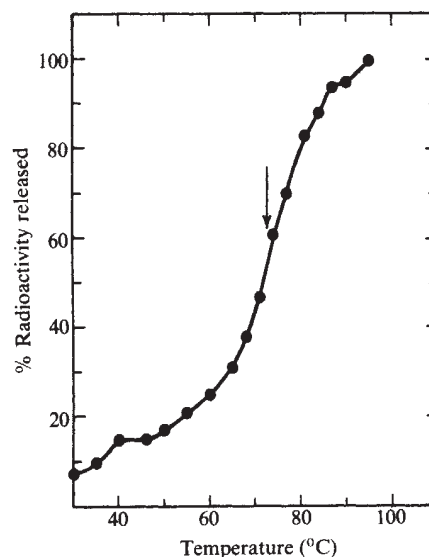


Fig. 2 Hybridisation of cytoplasmic polyribosomal fraction II tRNA to ctDNA. Fraction II tRNA was prepared from cytoplasmic polyribosomes as described previously¹. tRNA was labelled with ¹²⁵I to a specific activity of 1.7×10^7 c.p.m. per μ g tRNA and hybridised to 163 ng ctDNA. The level of hybridisation was at least five times above the background radioactivity bound to *E. coli* DNA. Data reflect net percentage of the DNA hybridised. ctDNA was saturated at 0.23% of the DNA, which is equivalent to 8 cistrons.

The aminoacyl acceptor species represented in fraction II tRNA from both total cellular tRNA preparations and cytoplasmic polyribosomes were determined (Table 1). A calculation of the relative abundance of individual tRNAs for 13 amino acids was carried out and is valid, as the aminoacylation reactions were carried out at subsaturating levels of tRNA and were saturated for each amino acid. As only 13 amino acids with comparable specific activities of ¹⁴C were tested, it is possible that additional aminoacyl acceptor species are present. However, analyses did not demonstrate aminoacylation for the seven additional amino acids with varying specific activities of ³H. The percentage of aminoacylation is limited by this condition. Alanine, arginine, glutamic acid, leucine, lysine and phenylalanine were incorporated when both preparations were

Fig. 3 Thermal denaturation of cytoplasmic polyribosomal fraction II tRNA-ctDNA hybrids. These hybrids were thermally denatured in standard saline citrate as described by Mielenz *et al.*³. The T_m in these conditions is 73 °C, with denaturation occurring between 55 and 90 °C.



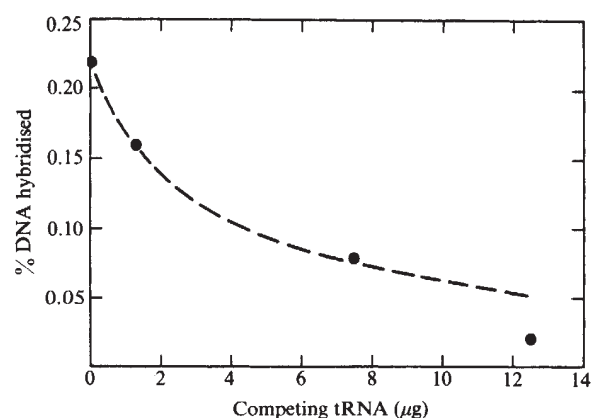


Fig. 4 Competition hybridisation between total cellular fraction II tRNA and cytoplasmic polyribosomal fraction II tRNA. Radioactively labelled cytoplasmic polyribosomal fraction II tRNA (1,250 ng with a specific activity of 1.7×10^7 c.p.m. per μg tRNA) was hybridised to 163 ng ctDNA in the presence of increasing amounts of nonradioactive total cellular fraction II tRNA. The points are the actual level of competition and the dashed line represents the theoretical level of competition.

aminoacylated. Glycine was incorporated into fraction II tRNA from cytoplasmic polyribosomes, whereas methionine was not. The tRNA^{met} is present at a level too low to drive the aminoacylation reaction, is absent in cytoplasmic polyribosomal fraction II tRNA, or is modified in a manner that inhibits aminoacylation. The same is true for tRNA^{gly} in the total cellular fraction II tRNA. Results were the same whether or not samples were deacylated before the reaction.

The fraction II tRNAs from mixotrophically grown cells and from cytoplasmic polyribosomes both complement 8 cistrons. A competition hybridisation with radioactively labelled cytoplasmic polyribosomal fraction II tRNA and nonradioactive total cellular fraction II tRNA was carried out to determine whether the same 8 cistrons are expressed in both cases (Fig.

4). The theoretical level of competition (dashed line, Fig. 4) was calculated assuming that approximately 75% of the competing tRNA, tRNA^{met}, was not present in the radioactively labelled tRNA (Table 1). Other variations in the relative abundance of different aminoacyl acceptor species in the two fractions exist, but this calculation takes into account the largest variation. The actual and theoretical levels of competition agree closely. Therefore, both preparations of tRNA complement the same cistrons of the ctDNA.

The present results together with those from our previous report¹, demonstrate that ctDNA codes for 8 distinct tRNA molecules in fraction II as isolated by chromatography on DBAE-cellulose; that isolated chloroplasts do not contain these fraction II tRNAs; that fraction I and fraction II tRNAs are coded by unique ctDNA cistrons; and that fraction II tRNA can be isolated from cytoplasmic polyribosomes. Transcripts of organelle DNA have not been identified previously at extraorganellar locations. These results may reflect the way in which a cell coordinates the synthesis of proteins in various subcellular compartments. For example, fraction II tRNA could participate in the cytoplasmic synthesis of proteins for the chloroplasts. Further, the difference in the relative abundance of various aminoacyl acceptor species in the fraction II tRNA may reflect a type of regulatory function for this tRNA. That selected species of tRNA are utilised in the synthesis of specific proteins has been reported⁷. Also, species of tRNA in cells change as new proteins are synthesised during development^{8,9}. The fraction II tRNA from *Euglena* may be involved in some analogous manner.

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Table 1 Aminoacylation of fraction II tRNA

Amino acid	pmol per μg tRNA		Relative abundance	
	Total cellular	Cytoplasmic polyribosomal	Total cellular	Cytoplasmic polyribosomal
Alanine	9.12	0.06	3.0	1.0
Arginine	0.18	3.30	6.5	52.6
Glutamic acid	0.12	1.27	4.5	20.3
Glycine	0	0.50	0	8.0
Isoleucine	0	0	0	0
Leucine	0.14	0.31	5.1	4.9
Lysine	0.14	0.07	5.1	1.1
Methionine	2.0	0	74.6	0
Phenylalanine	0.03	0.70	1.1	11.2
Proline	0	0	0	0
Serine	0	0	0	0
Threonine	0	0	0	0
Valine	0	0	0	0

Fraction II tRNA was isolated and aminoacylated as described previously¹. The activity with each individual amino acid was calculated as pmol amino acid per μg tRNA. All of the ¹⁴C-amino acids had similar specific activities. The pmol amino acid per μg tRNA for each amino acid was divided by the total pmol amino acid per μg tRNA of all the amino acids together. This calculation gives the relative abundance of each aminoacyl acceptor species of the mixture.

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matters arising

Association of young supernova remnants with γ -ray sources

MY original argument¹ that there are statistically unlikely coincidences of compact supernova remnants (SNRs) with the COS-B γ -ray sources² has been weakened by the recent discovery³ that one of the remnants classed as compact is not. The argument was based on the frequency of SNR 'hits' with the COS-B error boxes. A more rigorous study of the overlap between these two distributions can be carried out by calculating for each SNR the chi-squared value

$$\chi^2 = (\Delta l / \sigma_l)^2 + (\Delta b / \sigma_b)^2$$

where Δl and Δb are the differences in galactic longitude and latitude to the nearest γ -ray source and σ_l and σ_b are the respective standard deviations in the γ -ray source position. If σ_l and σ_b are equal to one another then χ^2 is the angular distance squared to the centre of the nearest γ -ray error box in units of the one-dimensional variance.

I have calculated χ^2 for each of the 51 SNR from the list of Ilovaisky and Lequeux⁴ which lie in the four regions surveyed in the COS-B observations. It is assumed that the errors quoted by the COS-B group are single standard deviations. (Application of the χ^2 formula to the two sources whose positions are known precisely, the Vela and Crab pulsars, gives χ^2 values of 0.56 and 2.1, which are reasonable for a χ^2 with two degrees of freedom. If, however, the error boxes are interpreted as being at the 90% confidence level, then these χ^2 values would increase by a factor of ~ 4 , indicating that the Crab position had an improbably large error associated with it.) With this assumption regarding the errors the probability that a source is actually found inside its own error box, 1σ on a side, is only 46%, and therefore the original analysis in terms of a hit or a miss of the error box is too restrictive.

The distribution of χ^2 for the 27 SNR with χ^2 values less than 20 is shown in Fig. 1. The dashed curve is the prediction of a Monte Carlo simulation assuming randomly distributed SNR. [The parameters of the distributions used in the Monte Carlo calculation were derived from an analysis of the 51 SNR. The latitude distribution was assumed to be gaussian with widths (σ) of 0.9° and 2.3° for the regions towards and away from the galactic centre respectively. The

longitude distribution was assumed uniform with a width of 40° except for the region near Cygnus in which the width was restricted to 69° to 94° to simulate the observed SNR clustering in this region.] The Monte Carlo calculation predicts 15.4 SNR with χ^2 values in the range of 4 to 20 in good agreement with the 13 observed to fall in this range. However, in the range from 0 to 2 the Monte Carlo calculation predicts slightly less than 4, whereas 10 are observed. The probability of a fluctuation of 10 or greater when the expected number is 4, is less than 1% on the basis of Poisson statistics. Therefore, the distribution suggests a statistically significant association of some (perhaps 6) of the remnants with some of the COS-B sources.

The 10 remnants (numbered according to the list of Ilovaisky and Lequeux) and their possible COS-B counterparts are: 40 with CG75+0, 42 with CG78+1, 65 with CG189+1, 87 with CG312-1, 93, 95, and 96 with CG327-0, and 99, 100, and 101 with CG333+0. The average diameter of these 10 remnants is 19.9 ± 2.0 pc whereas the remaining 41 have an average diameter of 30.3 ± 2.5 pc so that there seems to be a significantly smaller

diameter associated with those remnants which are near enough to γ -ray sources that there may be an identification. The age of a 'typical' SNR remnant as calculated from the Sedov relation quoted in my original paper for a diameter of 19.9 pc is 5,200 yr. Therefore, the suggestion advanced in the original analysis that there was an association of youthful SNR with some of the COS-B sources seems to be borne out in this more detailed analysis.

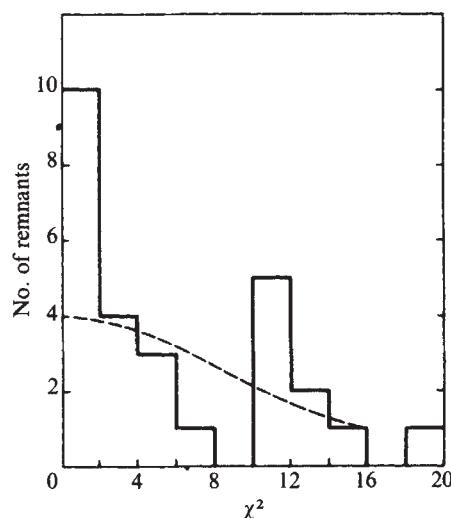
Of course this does not definitively prove anything, as we could be seeing statistical fluctuations, albeit improbable ones. The eventual decision on these possible associations must await future high resolution radio, X-ray, and γ -ray observations.

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Fig. 1 The distribution of SNR relative to the COS-B γ -ray sources. χ^2 is the sum of the squares of the latitude and longitude deviations of a SNR relative to the nearest γ -ray source. The dashed line gives the prediction of a Monte Carlo simulation assuming randomly distributed SNR. The excess of remnants in the first bin has a probability of less than 1% by chance.



Were Archaean continental geothermal gradients much steeper than today?

BURKE AND KIDD¹ have suggested that the scarcity of 'minimum-melting' granites in the Archaean Superior Province of Canada shows that temperatures at the base of the Archaean sialic crust (35 km depth) did not generally exceed 800°C . From this they deduce a surface geothermal gradient of less than 23°C km^{-1} compared with 17°C km^{-1} in such regions today. Although their suggestion for Archaean temperatures at 35 km depth is confirmed by P - T determinations on Archaean granulites (summarised in ref. 2) their calculation of the geothermal gradient does not comply with conductive properties and distribution of heat producing elements within the Earth. Their calculation merely divides 800°C by 35 km, assuming constant thermal conductivity, total absence of heat producing elements in the crust and constant geothermal gradient in the crust³. Such an extrapolation bears no relationship to a natural steady state geotherm and cannot be used

to infer a surface geothermal gradient. Their 'geothermal gradient' could only be produced by a sub-crustal heat flow of ~ 2 HFU (compared with modern values of 0.8 HFU) and no crustal heat production. A model geotherm for uniform distribution of heat production in the Archaean crust, fitting temperatures $\sim 800^\circ\text{C}$ at 35 km depth, gives a near-surface geothermal gradient of $\sim 40^\circ\text{C km}^{-1}$ (ref. 4). For a more geochemically reasonable, exponential decrease in heat production with depth in the Archaean crust⁵, an even larger near-surface geothermal gradient is obtained. The small amount of data available for Archaean P - T relationships suggests a surface heat flow at $\sim 3,000$ Myr, between two and three times modern values. This lack of data indicates the most urgent need for geothermal studies in Archaean terrains. Continental heat flow as high as that indicated by P - T studies has important implications for both lithospheric thickness and the dynamics of lithospheric motion that may not coincide with uniformitarian hypotheses about island arcs, Benioff Zones and so on, during the early history of the Earth⁶.

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BURKE AND KIDD REPLY—We did not intend to suggest that our gradients bore any relationship to near-surface geothermal gradients. It may have been clearer to have termed them average geothermal gradients. Our purpose in quoting these gradients was simply to emphasise that the temperature at the base of the crust (and therefore the contribution to conductive heat flow coming from below the crust) cannot have changed greatly since the stabilisation of the Archaean Superior Province greenstone-granodiorite terrains 2,500 Myr ago, and that this can be inferred from the lack of geological evidence for extensive melting of the lower crust in this and similar regions. Near-surface geothermal gradients are irrelevant to our arguments. We disagree completely that 'Archaean P - T relationships', presumably inferred from regional metamorphic mineral assemblages and their distribution, yield any information about the near-surface thermal gradients that existed in areas of Archaean continental crust in stable areas remote from orogenic zones. Such assemblages yield information only on the peak

thermal gradients during discrete events of metamorphism and orogeny. Very high, near-surface thermal gradients can be inferred from mineral assemblages in many regionally metamorphosed terrains of post-Archaean age; such thermal gradients are also only valid for the time and place of the orogeny, and contain no information about the near-surface heat flow through stable continental crust elsewhere at that time. Such orogenic and metamorphic events have been restricted to zones of plate convergence or arc/continental collision during the latter half of Earth history, and we see no reason to suppose differently for the Archaean.

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Deutsch's octave illusion

THE controversy over Deutsch's octave illusion can now be resolved. The disagreement was due to a difference in paradigm. Deutsch¹ used a dichotic sequence of 250-ms tones with no gaps between them. The tones were of equal amplitude and alternated in frequency between 400 and 800 Hz. The identical sequence was presented to both ears simultaneously; however, when the right ear received 400 Hz the left ear received 800 Hz, and vice versa. This repetitive sequence was presented continuously for 20 s. In these conditions the majority of listeners heard a single high tone in one ear alternating with a single low tone in the other ear. Further investigation showed that this effect was based on two factors: the following of the sequence of frequencies presented to one ear rather than to the other, and the localisation of each tone towards the ear receiving the higher frequency signal, regardless of which frequency was perceived². Further, right-handed subjects tended significantly to hear the high tone in the right ear and the low tone in the left (indicating a following of the sequence of frequencies presented to the right ear); however, left-handed subjects did not show this tendency.

Christensen and Gregory³ used a different paradigm. Subjects made pitch and localisation judgments concerning single, isolated dichotic chords. In these conditions neither the localisation effect nor the handedness correlate were obtained. However, Deutsch has shown that the localisation to the higher frequency signal occurs with long, repetitive sequences, and that with short sequences consisting of two dichotic chords localisation patterns closely follow patterns of relative loudness (see ref. 4).

Similarly, with long, repetitive sequences the handedness correlate described by Deutsch¹ is a clear and easily demonstrable phenomenon. However, Christensen and Gregory³ have shown that this does not obtain when judgments are made of single chords in isolation.

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Coenzyme A or adenosine inhibiting acetylcholine release?

COOK *et al.*¹ have reported that coenzyme A (CoA) inhibits the release of acetylcholine from autonomic nerves of the guinea pig ileum, and draw a comparison with other adenine derivatives such as adenosine, ATP and AMP which have been previously shown to reduce transmitter release in several tissues²⁻⁴ and are again shown to do so in their report.

However, in building their hypothesis on the role of CoA, the authors fail to discuss the possibility that the CoA added in their experiments may be metabolised to simple purine derivatives such as adenosine, which are the active materials. Such a metabolism to adenosine from extracellularly applied ATP, AMP and cyclic AMP seems to occur in other situations in which it has been examined^{5,6}.

This conclusion is further supported by the author's own observation that theophylline, a known antagonist at the adenosine receptor, would block the effects of all the purine derivatives tested, including CoA.

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COOK ET AL. REPLY—There is no doubt that some tissues possess the enzymes necessary to metabolise extracellular purine nucleotides to their equivalent nucleosides, so that there is the likelihood of adenosine being formed from any nucleotide which is based on the adenosine moiety. Although not specifically mentioned in our letter¹, it is likely that CoA gives rise to adenosine at some point in its breakdown and this might have been inferred both from the enhancement of the potency of CoA by dipyrindamole and by the demonstration of the efficacy of adenosine-3'-5'-diphosphate, a compound also likely to be formed during the breakdown of CoA. The problem, therefore, is not whether adenosine could be present but whether it is necessary. The comment seems to suggest that the ability of any purine nucleotide to reduce acetylcholine output might depend on the prior production of adenosine and that this would be the sole active agent. Although this could conceivably be the case it is unlikely that the receptor requires the nucleotides to be degraded to the nucleoside for agonist activity. Experiments using the ATP analogues α , β -, and β , γ -methylene ATP have shown² approximately equal potency to ATP, no difference in onset time, and sensitivity to theophylline. In addition, the offset time for these analogues was very much prolonged. It is unlikely that these compounds present a rapidly metabolisable substrate to 5'-nucleotidase and therefore it seems that the receptor, although referred to as the 'adenosine' receptor, may not require formation of the free nucleoside *per se*. It may recognise and also interact with the 6-aminopurine-9- β -D-ribose moiety wherever a compound contains it, provided that there is no steric hindrance preventing its presentation to the receptor.

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Diffusion-limitation of cell growth

THE paper by Whittenberger and Glaser¹ is entitled 'Cell saturation density is not determined by a diffusion-limited process'. Its refutation might similarly be entitled: 'The Stokes-Einstein equation should not be extrapolated to solutions of chain-polymers'. They assume that a 25-fold increase in bulk viscosity of a gel

must proportionately entail a 25-fold decrease in molecular diffusivity. This is incorrect both in principle (Stokes-Einstein was derived for homogeneous media) and in practice^{2,6}. For a small molecule like insulin the decrease in D would be nearer 25% than 25-fold.

Quantitative studies on diffusion require at least four parameters: D , Q , C and a penetration depth³. The authors¹ would have made a welcome start by measuring these.

Qualitatively speaking, diffusion-limitation is probably not the only limitation to cell growth in crowded conditions. But surely, even on a qualitative level, Occam's razor should be used? Thus it is necessary to eliminate the effect of stretch-limitation⁴, a phenomenon which has been independently demonstrated on single cells, and the possible interaction between stretch-limitation and diffusion-limitation for crowded cells in a gelled medium⁴ before proceeding to invoke yet a third hypothesis, namely contact inhibition¹.

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WHITTENBERGER AND GLASER REPLY—We thank Maroudas for pointing out to us a potentially very important reservation regarding our observations¹. As the applicability of the data cited by Maroudas² to our system is not clear³, we have measured in a synthetic boundary cell⁴ the diffusion coefficient of several proteins in the molecular weight range of potential growth factors in the presence and absence of the polymers used in our work on cell growth. The measurements were carried out in calcium and magnesium free Hank's solution buffered with HEPES⁵, and the results are shown in Table 1.

Although the effect mentioned by Maroudas is quite real, its magnitude is not as large as he anticipated. The effect of the polymers we used on the diffusion of small proteins is large enough for us to have observed an effect on the final cell density or on the rate of growth of the

cells if the growth of our cells was diffusion limited. Therefore, the conclusion of the paper is still valid. The reference by Maroudas to gelled medium is not clear as none of our media are gelled. Regarding the general comments of Maroudas with regard to contact inhibition of growth we would like to point out the following: Swiss 3T3 cells remain at constant density in spite of daily medium changes. In collaboration with D. Raben and M. Lieberman, we have shown that a plasma membrane-enriched fraction from 3T3 cells blocks cell growth early in G1, can bind to 3T3 cells on a dish, and mimics the effect of high density on the rate of uptake of smaller molecular weight nutrients. Membranes do not deplete bulk medium of growth factors⁵. The inhibitor activity from membranes has recently been solubilised from membranes (B.W., D. Raben, M. Lieberman & L.G., *PNAS*, in press); it is maximally active at levels of 10 μ g protein ml⁻¹ and is totally reversible. At this level it is unlikely to interfere with the surface available for cells to grow. We believe that in this instance Occam's razor suggests that one of the elements that controls cell growth in Swiss 3T3 cells is cell contact.

The original wounding experiments by Dulbecco⁶ clearly distinguish between the serum requirements at the wound and topo-inhibition, in agreement with the notion that cell to cell contact is involved in growth control. More recent experiments by Westermark⁷ on a human glial cell line and epidermal growth factor also agree with this notion.

All proteins were obtained from Sigma.

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Table 1 Diffusion constants of proteins in the presence of various polymers

	Buffer	+10% Dextran	+10% Ficoll	+2% Methyl Cellulose
Ribonuclease A	11.4	3.85	5.0	6.60
Soybean trypsin inhibitor	10.2	2.83	4.85	5.15
Pancreatic trypsin inhibitor	11.4	3.8	—	—

Diffusion constant cm² s⁻¹ × 10⁷.

reviews

Collaboration of equals

M. L. Oliphant

The Self-Splitting Atom: A History of the Rutherford-Soddy Collaboration. By T. J. Trenn. Pp. 175. (Taylor and Francis: London, 1978.) £6.

AFTER he had isolated heavy water by continued electrolysis, G. N. Lewis of Berkeley presented Rutherford with two tiny samples of the new and intriguing liquid. Experimental work in the Cavendish Laboratory with this and with the deuterium ions derived from it, and similar work in Berkeley, demonstrated the enormous power of these ions as projectiles for bringing about nuclear transformations. The Royal Society, of which Rutherford was President, decided to hold a seminar on heavy hydrogen, at which I was present. Rutherford opened the discussion with some general remarks, and the speakers were Aston, Sidgwick, Harteck, Fowler, Polanyi, Rideal, Bell, Bernal, Jevons, and finally Soddy. All previous speakers had spoken of deuterium as an isotope of hydrogen. However, Soddy, red in the face and obviously angry, said among other bitter words:

"... Further, it has been forgotten that this marked difference of chemical character that has been discovered for heavy hydrogen entirely destroys the prediction... I have never given a place in the periodic table to this element at all, and therefore regard the expression 'hydrogen isotope' as doubly a misnomer. It may prove that it is even easier to synthesise heavy hydrogen artificially than to disrupt the light elements... Heavy hydrogen bids fair, I think, to be one of the great discoveries of the century. I hope it may prove valuable not only in smashing up a few atoms, but even more so in smashing some of the physical theories of the present day about the whole of them."

Rutherford said a few soothing words, but Soddy was emotionally very upset, and clearly remained convinced that the word 'isotope', which he had invented, was being misused. This was the only occasion upon which I met Soddy, and it seemed to me that his ideas about atomic structure had atrophied since his great contribution to the collaborative work with Rutherford in Montreal thirty years earlier, and his great sequel to these investigations made in Glasgow. It seemed to me that Soddy must have been very much Rutherford's junior partner when, together, they opened up a whole new world of chemistry and physics

by disclosing the nature of radioactivity, and the spontaneous transformation of the heavy chemical elements. How wrong I was, is shown convincingly in this record of painstaking historical research by Thaddeus J. Trenn.

Trenn quotes from a letter of reference from E. Rutherford for F. Soddy, 15 January 1904, in which he says:

"... Mr Soddy throughout undertook the purely chemical work required in these investigations. He rapidly became acquainted with the physical methods employed in the measurements of radioactivity and in later work took a full share in the purely physical measurements."

"The work published by us was joint work in the full sense of the terms; for Mr Soddy not only assisted in the experimental work, but also supplied many of the suggestions and explanations incorporated in our published papers."

These are the words of an equal, not of a senior investigator about an assistant. It is clear that there was no claim by Rutherford to the limelight cast by their mutual discoveries. It is Trenn's purpose, in this thesis, to show why Rutherford's part in the great detective work which unravelled radioactive disintegration, changing the course of physical science, has dominated the history of the discoveries. In this he succeeds, changing that history into one of human relationships and characteristics, rather than only a part of the history of scientific discovery.

In any discussion of the Rutherford-Soddy relationship, it must not be forgotten that, although Rutherford was six years older than Soddy, he was a brash colonial boy from a small farm in New Zealand. He had been lucky in his schooling, and in having a mother who believed in learning, but these were no substitute for the English upbringing of Soddy, who was prepared thoroughly for Oxford and did not really need his scholarship to Merton College. Rutherford was extremely lucky to win an 1851 scholarship to Cambridge because the chemist, to whom it had been awarded, decided to remain in New Zealand and marry. Rutherford had served an apprenticeship in research at Canterbury College, but had to borrow the money for his fares to England. (I remember that when, in 1927, I reached the Cavendish Laboratory as a research student, I felt but half-educated beside the products

of English schools with a Cambridge degree.) So, although he began his work in the Cavendish Laboratory at the same time as Soddy entered Merton College in 1896, their backgrounds and intellects were not similar. Soddy remained always the cultured Englishman, though his Calvinist upbringing made him sometimes unsympathetic towards the feelings of others. As Fleck says in his Royal Society Biographical Memoir, 1957: "Truth as it was conceived was the essential thing. The method of its presentation, even at the expense of other people's feelings, was unimportant." On the other hand, Rutherford retained till his death the boyish charm, the enthusiasm, and the generous ways of the farm-boy from New Zealand, with an uncanny intuition, and a capacity for hard work, which seemed to have faded in Soddy after his Glasgow period. Having heard Soddy speak on two occasions, once at the Royal Society as I have already disclosed, and once at a public lecture in Cambridge on 'Monetary Reform', I cannot subscribe to the statements made both by Trenn, and by Feather in his foreword, that Soddy's command of the English language was much greater than Rutherford's. The conclusion that Soddy was a poor lecturer is supported by his record as such in Aberdeen, Glasgow and Oxford.

Trenn tells in detail of that remarkable period of cooperation between Rutherford and Soddy, in Montreal, from 1901-03. He seems to have entered, as the true historian should, into the minds of the two investigators, and has managed skilfully to dissect the contributions of the one from the other. In the work, each was both chemist and physicist, but each was dominant in his own domain.

It is fascinating to follow how, beginning with the evanescent and capricious behaviour of the radioactive gaseous emanation from thorium, the nature of radioactivity, and the concept of a radioactive series, were demonstrated. Discovery of the instability of many heavy atoms which, by spontaneous emission of a negatively charged electron, or a positively charged α particle, transmuted the atoms into totally different chemical elements, will remain always one of the most revolutionary of all discoveries. And it all emerged in less than two

years, from the combined efforts of two remarkably young men, a physicist and a chemist, the fourth son of a New Zealand farmer, and the seventh son of a successful London merchant. In his foreword, Professor N. Feather, who must be more deeply versed in general radioactivity than any other physicist in Britain, and a one time research student of Rutherford in Cambridge, writes of Trenn's analysis:

"He sees the collaboration of 1902-03 as the collaboration of equals: I am convinced that he is right". I agree. □

Sir Mark Oliphant collaborated with Rutherford from 1927-1937, was Professor of Physics at the University of Birmingham from 1937-1950, and was Director of the Research School in Physical Sciences at the Australian National University from 1950-1965.

Linear oceans

Waves in the Ocean. By P. H. LeBlond and L. A. Mysak. Pp. 602. (Elsevier Scientific: Amsterdam, Oxford and New York, 1978.) \$98.50; Dfl237.

IN the realm of ocean waves and circulation, as in most other physical systems, the system's behaviour can often be described to a good approximation by linear theory. In the ocean the approximation works best for waves in which the water particles move much more slowly than the waves themselves. Thus, it works well for tidal waves in the deep ocean.

The approximation also works for sea waves, the ones that give sea sickness, but here non-linearities can become important after a few hundred wavelengths. The approximation does not work well for internal waves or planetary waves. Internal waves are waves on the density surfaces within the ocean and planetary waves give the meso-scale eddies in the ocean, which correspond to the high and low pressure regions of the atmosphere. They dominate the currents over most of the ocean.

The linear approximation is also a good basis for a book, and it has been chosen by LeBlond and Mysak as a central theme in their excellent review of the theories concerning the complete spectrum of waves within the ocean. The authors approach the subject from the point of view of applied mathematicians and they take pains, and many pages, to show how the various approximations arise, how they are related, some of their pitfalls, and how they can lead to elegant analytical results.

They make qualitative comparisons between the models and the real ocean, but rarely go further. The approach and results of the more realistic numerical models are only briefly discussed, apparently because "no advantage is to be gained from approximations designed towards the application of analytical methods". But within the limits of the authors' self-imposed constraints, and the restricted understanding of the ocean it produces, the book is up to date and comprehensive. It contains 1,100 references.

Chapter one covers the equations of motion, the basic approximations and the general properties of plane waves. Wave action is introduced as an adiabatic invariant and is then used as fundamental quantity in the rest of the book. Chapters two and three review the various types of wave in a flat-bottomed ocean with simple stratification.

Chapter four introduces coastlines and includes good sections on the trapping of waves near to coasts and around islands. Chapters six and seven cover the interactions of waves with each other and their surroundings. There are sections on critical layer absorption and baroclinic instability

and an original comment that the results of Longuet-Higgins and Fox support Hasselmann's theory of the growth of the sea wave spectrum.

The final chapter is on the generation and decay of the waves. The section on surface waves is very up to date. It starts with Banner and Melville's recent work on flow separation and finishes with a comparison between experimental data and Gent and Taylor's numerical model. The section on tides is weak. It includes an interpolation between the diurnal and semi-diurnal tides without apparently realising that the forcing functions are orthogonal. The authors are honestly vague on the generation and decay of internal waves and conclude with another good section on coastally trapped waves.

The authors are to be commended on producing an excellent review of waves in the ocean, but they must be held partly responsible for the price, which puts the book beyond the reach of most readers.

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Molecular virology reviews

Molecular Biology of Animal Viruses. Vols 1 and 2. Edited by D. P. Nayak. Pp. 552 and 480. (Marcel Dekker: New York and Basel, 1977 and 1978.) \$45 each volume.

THESE two volumes on animal viruses are announced by the publishers to be textbook covering the major groups of animal viruses in a systematic fashion. Volume 1 begins with a chapter on virus architecture and another on interferon, followed by seven chapters on particular groups of RNA viruses: picornaviruses, togaviruses, rhabdoviruses, myxoviruses, reoviruses, and retroviruses. Volume 2 comprises five chapters on DNA viruses: parvoviruses, papovaviruses, adenoviruses, herpesviruses and poxviruses.

Most of the chapters are authoritative and clear accounts of the virus groups under discussion. But there are two major aspects of these volumes that lead one to question the wisdom and value of publishing such a book at all. The first is the rapid obsolescence of the reviews, a matter about which the editor is acutely aware in his preface. Nearly all the chapters were written in 1975 and although some have brief addenda citing major advances up to early 1977, too high a proportion of the chapters seem seriously dated already. A two- to

three-year incubation period between writing and publishing is not viable in this field today. Many of the authors apparently feel the same, as they have taken pains to state precisely when their chapters were completed.

Secondly, these volumes do not really represent a textbook, rather a series of unlinked reviews. Each of the chapters could equally well have appeared without alteration and much faster in one of the volumes of annual review publications that cover virological topics. So one must ask whether there is a particular advantage to grouping them into two volumes with the consequent delay in publication. The answer would be affirmative if the book represented a genuine textbook and included some general aspects of the molecular biology of animal viruses. But this is not the case; the student will gain little insight into the diversity of viral strategy nor the unity of virology as a discipline, without a chapter on the comparative molecular biology of viral replication and expression, or of pathogenesis. And important groups of animal viruses (arenaviruses, bunyaviruses, most insect viruses) are not mentioned. As a collection of special reviews the volumes will be useful to some virologists, but they cannot be recommended to those in search of a modern textbook of molecular virology.

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Transport processes in animals

Transport of Ions and Water in Animals. Edited by G. L. Gupta, J. L. Oschman, R. B. Moreton and B. J. Wall. Pp. 817. (Academic: London, New York and San Francisco, 1978.) £32.

THE thirty chapters in this book, each of different authorship and originating from several different countries, have been assembled as a tribute to Professor J. A. Ramsay who retired from the Chair of Comparative Physiology at Cambridge in 1976. Professor Ramsay has won great renown and respect as a pioneer in the development of microtechniques and in the application of these to the study of transepithelial transport, especially in insects. However, the contributions to this volume range widely over the animal kingdom, from amoebae to mammals and, as Professor Ussing observes in his prolegomena, the book could be regarded as a 'short-cut' to the ideas developed about transport processes from work on a wide variety of experimental preparations.

Among the contributions primarily devoted to techniques, there is an admirable review of the use of X-ray spectrometry as an adjunct to electron microscopy by Gupta, Hall and Moreton. This technique, though calling for great care in the preparation of specimens, offers the remarkable capability of revealing concentration gradients within single cells.

Renal physiology is represented by succinct yet balanced surveys of micropuncture and the perfusion of isolated tubular segments by G. Giebisch and W. H. Dantzler respectively. The vertebrate gall-bladder, which has proved so useful in the study of isotonic transfer, is discussed by T. Zeuthen and the fish gill, noted for its so-called "chloride cells", is the subject of a chapter by W. T. W. Potts and also receives some attention from L. B. Kirschner in the wider context of salt-excretion by marine vertebrates.

A rather general and mildly provocative evaluation of current models designed to elucidate the mechanism of salt/water coupling comes from the pen of A. E. Hill and includes a consideration of the possible involvement of electro-osmosis. Also of a rather general nature is the excellent account by N. J. Abbott and J. Treherne of homeostatic processes in the brain.

Intercellular junctions, predictably, figure prominently in many parts of the text but gap junctions attract a very detailed treatment in a beautifully illustrated and thought-provoking

article by W. J. Larsen, which suggests for them a special significance in hormone-responsive tissues. Like intercellular junctions potential measurements are frequently mentioned since many authors base their arguments very heavily upon them and the clear message comes through that electrophysiology is no longer the speciality of workers on excitable tissues.

Topics which are perhaps less central to the main theme of the book but which provide fascinating reading, are transpiration in arthropods, which is dealt with by E. B. Edney, and the uptake of water-vapour by insects and other small creatures, which is graphi-

cally described by J. Noble-Nesbitt. In addition there are more specialised articles on subjects which include the rectum and salivary glands of the cockroach, the excretory organs of larvae and the antennal gland of the crayfish.

For the generality of physiologists it may be fairly claimed that this book offers something for everyone and for most of them everything in it will be something worth reading. Unfortunately its cost will make it a luxury.

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Southern African biogeography and ecology

Biogeography and Ecology of Southern Africa. Vols 1 and 2. Edited by M. J. A. Werger. Pp. 1-660 and 661-1439. (Junk: The Hague, 1978.) DGu. 365.

To say that any attempt at condensing the biogeography and ecology of southern Africa into two volumes is a difficult task is a gross understatement. This being so, M. J. A. Werger has completed a courageous undertaking in editing a collection of review papers broadly dealing with this subject. He tackled the problem by devoting an introductory section to the physical and geological environment of the region, together with the history of its biota. There follows a series of eight chapters dealing with geographical subdivisions of southern Africa, and then one chapter on primary production, followed by a series in which animal classes are treated individually. Several rather special groups are dealt with separately, such as freshwater organisms, marine habitats and the vegetation of heavy metal contaminated soils. Finally, a chapter is devoted to the conservation of southern African ecosystems.

The idea of a physical and historical introduction is a sound one. The time element is given emphasis at a variety of scales. For example, an extensive account of the late Cretaceous and Tertiary vegetation history is provided by Axelrod and Raven, who draw upon a fund of recent work in plate tectonics to produce the first major synthesis on this subject for 25 years. For the general ecologist this may rate as the high point of the book.

Van Zinderen Bakker continues with a sadly brief account of Quaternary vegetation changes. Generalisations are made concerning the vegetation zones of "a glacial maximum" and "an interglacial", but no indication is given

concerning the number of sites for which pollen data is available and their geographical locations. Neither is any pollen diagram presented. More detail should have been given to this part of southern Africa's history, the understanding of which is vital for any assessment of the present or future states of vegetation in the area.

Short term climatic variations are dealt with in a paper by Tyson, who describes 10- and 20-yr rainfall cycles, based on meteorological records and dendrochronology. Such data is of importance in understanding the role of climate and land use in the recent desertification of areas previously covered with grass.

The remainder of the first volume concentrates upon the phytogeography of this varied area. Each part of the region has its own interest, from the exceptional floristic richness of the cape region (some 100 m² quadrats may contain more than 120 angiosperm species) to the open communities of the Namib desert. These accounts are basically descriptive, but attempts are made to relate the communities described to the major environmental factors controlling their composition and distribution. The impact of grazing and fire is discussed, but little information seems to be available concerning secondary succession in the area. The arrangement of the animal data in a taxonomic rather than biogeographical form is unfortunate in that one loses sight of the animal-plant relationships as a consequence. It was, however, probably unavoidable, as different authors are responsible for each animal group.

The two volumes form a valuable body of reference on which more data will accrete. As with preceding volumes in this series, their appeal is mainly limited to those with research interests in the geographical area concerned.

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Fungal miscellany

Fungi: Delight of Curiosity. By Harold J. Brodie. Pp. 131. (University of Toronto Press: Toronto, Buffalo and London, 1978.) \$10.

Dr Brodie is a distinguished mycologist who has devoted much of his life to the biology and taxonomy of the beautiful bird's nest fungi (Nidulariales). Now in retirement he has attempted to convey his delight in fungi generally to others in a series of short and rather philosophical essays. Clearly he has enjoyed writing this book and, perhaps because of that, it is easy to read; and being so short, it can be read in an evening.

The essays deal with a number of separate subjects, mainly mycological oddities. These subjects are: the gunnery of *Pilobolus*; the nature and growth of the fungal mycelium; form and function in gill-bearing toadstools; the elegance of the coral fungus (*Hericium coralloides*); the splash-cups of his own bird's nest fungi; fungi in soil, with special reference to those that capture eel-worms; the remarkable gasteromycete *Sphaerobolus*; Laboulbeniales under the title "insect itch"; the attine ants and their fungus gardens; and finally a mushroom monstrosity, namely the toadstool *Lactarius* parasitised by the ascomycete *Hypomyces lactifluorum*. The book is a fungal miscellany.

The author refers at the outset to the well known biological fable of the three blind men and the elephant. "Each man developed his own mind picture according to the nature of his particular contact with the elephant. The blind man who touched the sinuous and writhing trunk thought of a snake; he who took hold of the firm tough tail thought of a rope; and he who felt the rough and massive leg pictured the base of a tree." This theme of the limitations of partial knowledge recurs throughout the book, and so do the blind men. In the final paragraph the author remarks: "In retrospect I would conclude that the three blind men do not know very much about fungi after all." We mycologists are the blind men.

Each of the essays is illustrated by up to three photographs. There are no line drawings. The pictures vary in

quality. A few are excellent, but some are most disappointing—a pity as the whole emphasis of the book is on the beauty of fungi. The figure of *Pilobolus*, one of the loveliest moulds, is quite good, but it is disturbing to read in the legend that the spores are shot "on to vegetation where they will grow to create new plants." Happily in the text the true story is told.

In the essay on splash-cups, *Polyporus conchifer* is described but not figured. However, there is a full-page picture of *Laeticorticium minnsiae* without any reference to it in the text. Incidentally splash-cups are called "water-guns", surely an odd term. During washing up when one's face is splashed by the jet of water from the tap being reflected from a spoon in the sink, it would hardly seem appropriate to refer to the spoon as a gun. Surely in a gun the propulsive force is generated within the weapon?

Treatment of individual subjects is sometimes so generalised that a vital point is missed. In the chapter on form

and function in toadstools the essential fact that the range of the basidial guns is less than the distance between the gills is not mentioned. Further, in a spore-print the spores are said to "pile up on the paper in lines corresponding to the position of the thin spore-plates", whereas the lines actually correspond with the spaces between the gills.

It is difficult to estimate what impression the essays would make on someone without mycological knowledge, although the author seems to have in mind "persons who have had little contact with basic concepts of biological science." Certainly the book is not for the student or for the dedicated naturalist, as the information conveyed is not clear-cut and comprehensive. Perhaps those who might most enjoy it are fellow mycologists who would know the background and would appreciate the philosophical asides.

C. T. Ingold

C. T. Ingold is Emeritus Professor of Botany in the University of London, UK.

Contamination of the environment

Air Pollution. Vol. 2: The Effects of Air Pollution. (Third edition.) Edited by A. C. Stern. Pp. 684. (Academic: New York, San Francisco and London 1977.) \$43.50; £30.90.

ARTHUR Stern is a well-known and much respected elder statesman in the field of air pollution and his collections of review articles, now grown from two volumes in the first edition of 1962 to five volumes in the present third edition, have become for the environmentalist almost what Grove is to the musicologist. The proliferation in recent years of published work and opinions concerning the contamination of the environment is truly frightening, especially as much reported work is so bad that it would never have seen the light of day had editors and referees done their jobs. There is a need, therefore, for an authoritative work on this massive topic and Stern's set comes as close as any other publication to being the standard text on the subject.

Volume 2 of the third edition is concerned with the effects of air pollution and is much larger than its predecessor (concern about air pollution would seem to be inversely proportional to its concentration), and is the product of 14 distinguished contributors. Part A deals with effects of pollution on physical and economic systems and part B is concerned with the appraisal of effects on biological systems. Part

C is a welcome bonus on air pollution literature resources which is a useful if daunting guide to reports (current and past), journals and other literature. Not surprisingly, it is already a little out of date. Another welcome feature is the inclusion, before the text, of the contents of the other volumes.

In part A there is a timely chapter on the effects of pollution on the quality of air indoors. Part B deals with effects on vegetation, biological effects (mainly an attempt to review the reported effects of specific pollutants on animal models) and the effects of pollutants on human health. There is a section on chemical analysis and bioassay for carcinogenicity which could be said to take up too much space compared with that allotted to the section on human health.

To attempt to review in detail a book of this size and density would be an impossible task. Suffice it to say that there is much sound discussion, though the opportunity to be critical enough to purge the literature of some of the notoriously slipshod work has often been lost. A volume in which fourteen authors have written must necessarily take much time to edit and compile, so that the contents are accordingly behind the times; references later than 1975 are rare.

The book and its companion volumes merit a place on the shelves of those authorities who have responsibilities to protect the environment.

P. J. Lawther

P. J. Lawther is Director of the MRC Toxicology Unit (Clinical Section) at St Bartholomew's Hospital, London, UK.

● In the review of *The Story of Cancer* (Nature 27 July; 274, 404, column 3, lines 18–20 should read "... malignant human neuroblastoma cells can be induced to differentiate terminally into mature neurones, and erythroleukaemia cells can be induced to differentiate terminally into RBCs, and how particular ...").

obituary

P.C. Sylvester-Bradley

PETER COLLEY SYLVESTER-BRADLEY, the F. W. Bennett Professor of Geology at the University of Leicester died unexpectedly at Weston-Super-Mare on 17 April 1978, at the end of a student vacation field course, at the age of 64. Tragically his death came only five months before he was due to retire and continue with his researches in micropalaeontology, fossil oysters and evolution—all subjects in which he had attained international eminence. With his death, British palaeontology has lost one of its most distinguished scholars.

He was born on 21 May 1913 at Pinhoe, Devon and went to Haileybury College, Herts. where he was a science prize winner. He graduated in geology at the University of Reading where he cultivated a range of other scientific interests: for example he became the first editor of the *Proceedings of the Reading and District Natural History Society*. He started his geological research on the Purbeck Beds of Dorset and became interested in their great variety of ostracods, a neglected group of microfossils on which he was later to become one of the world's leading authorities.

His first appointment was to found a geology department at the Agricultural College of Seale Hayne in Devon. This was interrupted by the second world war and, after a brief period at the British Museum, he joined the Royal Navy. After the war he became a lecturer, later senior lecturer, in palaeontology at the University of Sheffield, except for the year 1955-56 when he was Rose Morgan Professor at the University of Kansas. In 1959 he became the first F. W. Bennett Professor of Geology at the University of Leicester, a post which he held with great distinction. He built up a department which now has a high international standing in the major branches of geology and which has produced a number of eminent scholars. He took a personal interest in all the activities of his department and his scientific interests consequently broadened considerably.

His principal field of interest was in micropalaeontology, and in particular the internal and external structures of ostracods. These studies took a great leap forward when he applied

the newly-invented scanning electron microscope to their study. This provided a considerable amount of new data of high quality which he kept improving by careful experimentation. His work was published in the form of a stereo-atlas of ostracod shells, a new and improved form of palaeontological publication. He applied his expertise on ostracods to the history of the Mediterranean and to enhance our understanding of the Jurassic geology of Britain.

Another major life-long interest was the Jurassic oysters from Europe and to some extent from North America, from which he developed his ideas on iterative evolution. He wrote and lectured extensively about aspects of evolution ranging from the origin and evolution of life to the evolution of ethics. He contributed a great deal to the ideas on the volcanic environments in which life may have originated and directed attention to the possibility of evidence for the earliest forms of life being found in low-grade metamorphic areas. The geology of carbon and the origin of oil were related interests. He was a leading figure in the world of systematics and wrote extensively about the numerical approach to taxonomy, the concept of species, subspecies, superspecies, form genera, diversity and stratigraphic nomenclature.

He was active in over twenty scientific societies. At various times he was Chairman of the British Micropalaeontological Society, President of the Systematics Association, Vice-President of the International Palaeontological Association and of the Palaeontological Association. He also served as editor of a number of palaeontological and geological publications.

Peter Sylvester-Bradley was a brilliant lecturer and was always in great demand. His lectures on a wide range of subjects, were always very vivid and generally contained the latest ideas which stretched his students and staff to the limit. He had very wide interests outside the field of geology. He was a keen landscape gardener. He maintained active interests in conservation, travel, country wines and the arts, particularly operatic music. He was well-known in Leicester through his activities with the Haldane Society, The Leicestershire Trust for Nature Conservation and the Leicester Literary

and Philosophical Society, over all of which he presided. A devout Christian, he was a regular lay-preacher at the Stoughton village church. He was regarded with the greatest affection by all members of his department and will be sadly missed by his colleagues throughout the University and friends in many parts of the world. He leaves his wife, Joan, a fellow student at Reading whom he married in 1945, a daughter, Rosemary, and three sons, Rowan, Roger and Benjamin.

M. A. Khan

Sir Harry Jephcott

SIR HARRY JEPHCOTT, who died on 29 May 1978, at the age of 87, played a very important role in the development of the British pharmaceutical industry and in the applications of science to industrial needs.

Born at Redditch in 1891 he graduated in chemistry in 1915 and worked in the Laboratory of the Government Chemist to earn sufficient to enable him to study and qualify as pharmaceutical chemist in 1916. In 1918 he joined Glaxo and there began a remarkable career first as chemist then as General Manager, Managing Director, Chairman and Managing Director of an organisation originally manufacturing and marketing baby foods and developing to an organisation which, on his retirement in 1963 was the major producer in this country of penicillin, streptomycin, cortisone, hydrocortisone, poliomyelitis vaccine and other immunological products. It is a remarkable development reflecting the skill and influence of a remarkable man.

In his book *The First Fifty Years*, published for private circulation in 1970, Jephcott recalls the history of the Nathan organisation and the beginnings of the pharmaceutical interests of Glaxo. But it was the years of the Second World War that revealed Jephcott's potential. First as manufactured foods adviser to the Ministry of Food he brought a new concept to the definition of composition, description and labelling of manufactured foods that had the encouragement throughout of Lord Woolton. Simultaneously Jephcott found time and energy to direct the participation of Glaxo in the development of manufacture of peni-

collin; first by "bottle culture", then by deep fermentation.

It is a fascinating story of confidence in the ability to solve technical problems while being a realist in the use and need of capital resources and human skills. Initially through collaboration in the Therapeutic Research Corporation, but subsequently as agent for the Ministry of Supply, Jephcott secured agreement with the Merck, Squibb and Pfizer organizations in the USA which in the late 1940s enabled the manufacture of penicillin by deep fermentation to be preferred to the "tray" methods being explored by ICI and the small container methods preferred by others. He recognised the potential help of mycologists as well as chemists and the importance of selecting strains and producing mutants, the metabolism of which in media fortified by phenylacetic acid and hydroxyphenylacetic acid increased the yields many fold. New factories were developed both for manufacture of bulk antibiotics and also for sterile filling into containers for individual use on patients, both in this country and in several countries of the Commonwealth. The manufacture of penicillin was accompanied within a few years of its inception by the manufacture of streptomycin and there followed in the 1950s the added bonus of production of vitamin B₁₂ from the fermentative processes of streptomyces species.

In the war years and subsequently, Excess Profits Tax limited the generation of financial resources for reinvestment and expansion, but with the abolition of EPT in the early 1950s the fortunes of the Glaxo organisation became transformed and deservedly so. For Jephcott had a fantastic intuitive sense of what was possible by industry; he could define the right course to take and rationalise his decision afterwards. He understood how to use wisely the expertise of others and never ceased to emphasise the possibilities of application of science. He could recognise and encourage capacity in applied chemistry and therein lay much of his success.

His example of hard work, devotion to duty, economical use of time and energy, fearless approach to challenge influenced many with whom he worked and dealt both in this country and overseas. The world became his parish and he travelled to all the continents developing local manufacturing units as necessary. He also served for five years as Chairman of the Department of Scientific and Industrial Research where his experience led him to advise Ministers of the different requirements for development of science and technology. Many in the research associations have good cause to be grateful for Jephcott's influence on their develop-

ment, but so have the professions of chemistry and pharmacy.

He was President of the Royal Institute of Chemistry in 1953-1955, Chairman of the Association of British Chemical Manufacturers in 1947-1950, an Honorary Auditor for 40 years of the Pharmaceutical Society from whom he received their Charter Gold Medal in 1970. He was Chairman of the Council of the School of Pharmacy, University of London, from its inception as a body independent of the Pharmaceutical Society in 1948 until 1969.

From his industrial successes he created several charitable trusts, but only in his latter years did he really learn to spend any money on himself. He was always generous to others but the hardships of his youth had taught him to waste nothing. Never a patient man he learnt of late to endure physical pain with fortitude and to the end he rejoiced in his friendships. He was a towering example of an upright man in every sense.

His services to the Ministry of Food were recognised by a Knighthood in 1946 and his transformation of the pharmaceutical and fine chemical industries by a Baronetcy in 1962. He left for others to write *The Second Fifty Years* of the organisation with which he became identified and to which he gave so much of his energy and life. It will be a fascinating story of the influence of intuitive judgment, recognition of the potential applications of science and a financial acumen that deserved success and achieved it.

Frank Hartley

G. H. M. Lawrence

PROFESSOR George Hill Mathewson Lawrence died at his home on Rhode Island in the United States on 11 June 1978. Born on 19 June 1910 at East Greenwich, Rhode Island, he studied at Rhode Island State College and Cornell University where he received his PhD in botany in 1939.

He helped the eminent taxonomist, Liberty Hyde Bailey, in the revision of his *Manual of Cultivated Plants* (1949) while he was his assistant at the Bailey Hortorium at Cornell from 1945 to 1950. Cornell appointed him a professor of botany in 1947 and four years later Lawrence became Director of the Bailey Hortorium. His *Taxonomy of Vascular Plants* (1951) which considered many aspects of the botanical sciences, especially phylogeny, was well received and was reissued in an abridged version in 1955.

In August 1960 he left Cornell to take up the post of Director of the Hunt Botanical Library (now the Hunt Institute

for Botanical Documentation) at the Carnegie Institute of Technology (now the Carnegie-Mellon University) at Pittsburgh.

The Hunt Library was already renowned as an outstanding collection of choice books, drawings, prints and portraits in the fields of botany and horticulture, and it being the earnest wish of its founder, Mrs Rachel McMasters Miller Hunt, that it should not be dispersed, it was presented to the Carnegie Institute of Technology. Lawrence's assessment of Mrs Hunt as a person of 'great stamina, of amazing vigour and dynamism' could equally apply to himself. It was his energy, enthusiasm and vision that transformed this private library into an international centre for biobibliographical research. He shared Mrs Hunt's concern for exacting standards and quality and all the library's publications are distinguished by impeccable scholarship and elegant design. Lawrence's most ambitious project was the *Bibliographia Huntiana*, an exhaustive bibliography of botanical and horticultural books and periodical articles published during the period 1730 to 1840. Although the *Bibliographia Huntiana* has not yet appeared the *Botanico-Periodicum-Huntianum* (1968) and the *Master Book List* (1972) are useful byproducts of the data already accumulated on magnetic tape.

Lawrence made substantial additions to the book stock, formed the nucleus of an archives collection and diversified the collection of botanical art with examples of the work of many contemporary artists. The acquisition, however, which gave him the greatest pleasure was Dr Birger Strandell's collection of books, periodicals and newspaper cuttings relating to Linnaeus. With Dr Strandell's assistance he undertook the compilation of an annotated catalogue of this immensely important collection and only a month before his death attended a conference in Sweden to report on its progress. Although he has not lived to see its publication, Professor Lawrence died as he would have wished, active and productive to the end.

Ray Desmond

James Shields

JAMES SHIELDS, who died on 20 June 1978, had achieved an international reputation for his contributions to the genetics of mental disorder and to twin research.

He was born on 21 November 1918. After two years at Oxford he was conscripted into the Royal Artillery. In 1945 he came back from five years in a prisoner-of-war camp with a fine record for helping to maintain morale. He came to psychiatric genetics at the Maudsley Hospital in 1947, and later was seconded for ten

years to the Medical Research Council's Unit. When that was closed in 1969 he returned to the Institute of Psychiatry, becoming Reader in Psychiatric Genetics.

During field work in 1954 he contracted polio, and thereafter was confined to a wheelchair. But with the courage that was typical of him he re-organised his life to surmount disablement and to continue research, lecturing, foreign visits and collaboration with a succession of visiting guest workers, virtually unimpaired.

He built up a register of consecutive cases of twins who were patients of the Maudsley Hospital, which has been, and it is hoped will continue to be the foundation of important field studies. The rigorous objectivity and self-critical understanding which he applied to twin studies has maintained it as a uniquely informative research tool after a period in which it was misunderstood and disparaged. His classic work on *Monozygotic Twins Brought up Apart and Brought up Together* was published in 1962. It was based on the largest collection of separated twins so far made; and his analysis threw new light on the role of genetics in social environment and upbringing.

Shields collaborated in many papers with Irving Gottesman. Their work on Schizophrenia and Genetics, reported in 1972, was awarded the Hofheimer Prize. After the most critical examination of the genetical hypothesis, it was found conclusively that genetic factors specific to schizophrenia are involved in its aetiology.

Shields's wide range of genetical work also covered anxiety states, neuroses, homosexuality, alcoholism and adoption in relation to schizophrenia. His knowledge of the literature was encyclopaedic; his scholarship, intellectual distinction and passion for truth in a contentious field were recognised by the (rare) award of the honorary D.Med. of the University of Zurich in 1975.

Unassuming, uncomplaining and generous, he endeared himself to his colleagues. He had, through thick and thin, the loving support of his wife Elizabeth, sharing with her a gift and an enthusiasm for music.

Eliot Slater

William Ellison

WILLIAM ELLISON, Professor of Agriculture at the University College of Wales, Aberystwyth, died a few months before retirement, on 11 April 1978, aged 66.

He was a graduate of King's College, Newcastle, where, in 1934, he also gained a Ph.D. for work on the cytology of potatoes. In the same year he was appointed assistant lecturer in agricultural botany at Aberystwyth. Until the outbreak of war he continued his cytological work and col-

laborated fruitfully with the staff of the Welsh Plant Breeding Station on the cytology of *Avena* species.

In 1939 his work and interests changed abruptly when he was appointed Chief Technical Adviser to the Montgomeryshire Welsh Agricultural Education Committee. This work, which continued throughout the war, was to bring him much renown, not only locally but nationally. He was instrumental in extending and putting into practice on a large scale the knowledge gained from experimental work of the 1920's and 30's, at the Welsh Plant Breeding Station and elsewhere, for the improvement of hill and marginal lands in Britain. His achievement confirmed not only his authority and expertise, but also his initiative and powers of leadership in this field.

When the war ended he became Professor of Agriculture at Aberystwyth and, from that time onward, he served his College with enormous dedication and the utmost loyalty. He served as Dean of the Faculty of Science and was Vice Principal from 1965 to 1967. He was a member of the U.G.C. sub-committee on agriculture from 1965 to 1975 and President of Section M of the British Association for the Advancement of Science in 1966. Apart from these 'academic' commitments Professor Ellison's influence was important in determining post-war agricultural policy towards the use and development of hill and marginal lands in the U.K., notably through his book on marginal land and as member of the N.E.R.C. Land Use Research Committee.

Professor Ellison was a staunch, even passionate advocate of the merits of university degree courses in agriculture. He would argue that the blend of science and technology, of art and craft, of economics and husbandry was a firm foundation for a broad, yet rigorous education. He would argue, also, that agriculture had as much to do with a way of life as with making a living. This is not surprising. He came from an ancient family of distinguished farmers, land agents and lawyers in the north country. He was proud not only of their professional achievements and enterprise but of the qualities of reliance, courtesy and public spirit which were part of their rural scene. Professor Ellison himself was well endowed with these qualities. He was unfailingly courteous, modest and unassuming and with an enormous capacity for making, and keeping, friends. These many friends will mourn his passing.

He is survived by two daughters. His wife died only four days before him, on 7 April 1978.

H. Rees

J. G. Valatin

JOHN GEORGE VALATIN, Professor of Theoretical Physics at Queen Mary College in the University of London, died on 19 April 1978, aged 60. Born in Budapest, John Valatin studied engineering at the Technical University there. Having obtained a doctorate for work on molecular spectra he worked for two years in industry before returning to the University at the end of the war as a lecturer in physics.

In 1947 he left Hungary to work with Louis de Broglie at the Institut Henri Poincaré. He was awarded the D.Sc. of the University of Paris for a thesis on the theory of the positron. In 1950 he went to the Niels Bohr Institute in Copenhagen, where he developed a covariant gauge independent formulation of quantum electrodynamics.

Two years later Valatin's interest in physics led him to the Mathematical Physics Department headed by Professor Peierls at Birmingham University. He spent thirteen years there and became a British citizen. In his early years in Birmingham he continued his studies in quantum electrodynamics doing pioneering work on the use of the point splitting technique to remove the divergence of that theory.

Following the fundamental work of Bardeen, Cooper and Schrieffer, and stimulated by the presence in Birmingham of J. R. Schrieffer as a young post-doctoral fellow, Valatin discovered in 1957 (independently of academician N. N. Bogolyubov) the canonical transformation which bears their names. This discovery gave greater insight into the nature of the ground state and of the elementary excitations in superconductivity. Over the next few years Valatin developed a generalization of the Hartree-Fock method designed to take account of the pairing forces which occur in superconductors. In collaboration with B. R. Mottelson and D. J. Thouless he used this generalized Hartree-Fock method to discuss pairing forces in nuclei and their influence upon nuclear rotational states.

In 1965 he left Birmingham for a chair at Queen Mary College, London, where he established a theoretical group working both in the theory of elementary particles and in the theory of condensed matter. He established a research seminar that drew distinguished speakers from all branches of theoretical physics and which served as an eloquent witness to his belief that theoretical physics was a unified subject.

Although John Valatin's life was dominated by his interest in physics, he was a deeply committed Christian and was much concerned with the welfare of students, especially those from overseas.

He is survived by his wife and two sons.

*R. B. Jones
W. Young*

announcements

Meetings

20 September, **Materials Selection in Food Processing**, London (The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

20–21 September, **Safety Aspects of Instrumentation**, London (Beverly Humphrey, Scientific Symposia Ltd, 33/35 Bowling Green Lane, London, EC1, UK).

20–23 September, **Optics '78**, Bath (Mrs D. L. Harmer, Royal Greenwich Observatory, Hertsmonceux Castle, Hailsham, Sussex, UK).

20–22 September, **Complex Metallurgy 1978**, Clausthal-Zellerfeld (The Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

24–29 September, **29th Annual Session American Association for Laboratory Animal Science**, New York (AALAS, Dr Alfred G. Edward, Wayne State University, 540 E. Canfield, Detroit, Michigan 48201).

25–27 September, **The Immune System: Function and Therapy of Dysfunction** (Seroni Symposia, Via Casilina, 125, 00176 Rome, Italy).

25–27 September, **The Skin of Vertebrates**, London (Linnean Society of London, Burlington House, London W8, UK).

25–27 September, **AGU Midwest Meeting**, St Louis (AGU, 1909 K St. N.W. Washington D.C. 20006).

25–28 September, **6th International Symposium on the Chemistry and Biology of Pteridines**, La Jolla (G. M. Brown, MIT, Cambridge, Massachusetts 02139).

25–29 September, **12th International Symposium on Chromatography**, Baden-Baden (Gesellschaft Deutscher Chemiker, Geschäftsstelle, Abteilung Fachgruppen, Postfach 90 04 40, 6000 Frankfurt/Main 90, FRG).

25–29 September, **Trends in Physics**, York (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

25–29 September, **3rd International Congress on Noise as a Public Health Problem**, Freiberg (Jerry V. Tobias, Institut für Arbeits- und Sozialmedizin Universitätsklinikum, Johannes Gutenberg Universität, Obere Zahlbacher Strasse 67, D-6500 Mainz, FRG).

25 September–1 October, **Palaentological Association**, Maastricht (DR D. G. Montagne, Natuurhistorisch Museum, Bosquetplein 7, Maastricht, The Netherlands).

27–29 September, **Wave and Tidal Energy**, Canterbury (BHRA Fluid Engineering, Cranfield, Bedford, UK).

28–29 September, **AGU 25th Pacific Northwest Regional Meeting**, Tacoma (Z. F. Danes, University of Puget Sound, Tacoma, Washington 98416).

Reports and publications

UK & Ireland—July

The Computer and Society. By Tom Crowe and John Hywel Jones. Pp. 16. (Fabian Tract 457.) (London: The Fabian Society, 11 Dartmouth Street, SW1, 1978.) 50p. [247]

Annual Report for 1977 of the Medicines Commission, the Committee on Safety of Medicines, the Veterinary Products Committee, the British Pharmacopoeia Commission, the Committee on the Review of Medicines, and the Committee on Dental and Surgical Materials. Pp. 122. (London: HMSO, 1978.) £2 net. [247]

Report of the General Practice Finance Corporation for the period 1st April 1977 to 31st March, 1978. Pp. 16. (London: HMSO, 1978.) 45p net. [247]

The Pharmaceutical Industry and the Nation's Health. Pp. 30. (London: The Association of the British Pharmaceutical Industry, 162 Regent Street, W1, 1978.) [247]

Research Strengths of Universities in the Developing Countries of the Commonwealth. Pp. 208. Second edition. (London: Association of Commonwealth Universities, 36 Gordon Square, WC1, 1978. Published in association with the Commonwealth Secretariat.) £3.50/\$8.50. [247]

Ministry of Agriculture, Fisheries and Food. Agricultural Development and Advisory Service Annual Report 1977. Pp. 99. (London: HMSO, 1978.) £4.50 net. [247]

Royal Observatory, Edinburgh. Annual Report, 1976–77. Pp. 60. (Science Research Council.) Pp. 60. (Edinburgh: The Director, Royal Observatory, Blackford Hill, 1978.) [247]

Office of Population Censuses and Surveys. Mortality Statistics: Cause. (Review of the Registrar General on Deaths by Cause, Sex and Age, in England and Wales, 1976. Series DH2 No. 3.) Pp. viii+105. (London: HMSO, 1978.) £2.75 net. [247]

Department of the Environment and Department of Transport Library Services. Annual List of Publications 1977. Pp. 126. (London: Departments of the Environment and Transport Library Services, 1978. Available from the DOE/DTp Sub-Library, Bldg., 6, Victoria Road, South Ruislip.) £1 (plus postage). [257]

British Nuclear Fuels, Ltd. Seventh Annual Report and Accounts, 1977/78. Pp. 33. (Risley, Warrington: British Nuclear Fuels, Ltd., 1978.) [257]

Grants to Local Authorities for Countryside Management Projects. Pp. 8. (Cheltenham: Countryside Commission, Crescent Place, 1978.) *gratis*. [267]

The Royal Society. Pollution in the Atmosphere: Final Report of a Royal Society Study Group. Pp. 25. **The Future Role of the Ordnance Survey: a Submission to the Ordnance Survey Review (Serpell) Committee by the Council of the Royal Society**. Pp. 60. **Long-term Toxic Effects: a Study Group Report**. Pp. 15. (London: The Royal Society, 1978.) [267]

National Coal Board. Report and Accounts 1977/1978. Pp. 88. **Statistical Tables 1977/1978**. Pp. 12. (London: National Coal Board, Hobart House, Grosvenor Place, 1978.) £1 net. [267]

Department of the Environment. Building Research Establishment Current Paper 30/78: The Ignition and Burning Characteristics of Fabric Covered Foams. By W. D. Woolley, S. A. Ames, A. I. Pitt and K. Buckland. Pp. 19. (Garston, Watford: Distribution Unit, Building Research Establishment, 1978.) *gratis*. [277]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 289, No. 1364: **General Connection Formulae for Liouville-Green Approximations in the Complex Plane**. By F. W. J. Olver. Pp. 501–548. (London: The Royal Society, 1978.) UK £3.85; Overseas £4. [277]

Tetrahedron Reports. No. 31: **Introduction of Fluorine into Organic Molecules—Why and How**. By M. Schlosser. Pp. 18. No. 32: **Organopalladium Intermediates in Organic Synthesis**. By Barry M. Trost. Pp. 35. No. 33: **Recent Developments in Sulfone Chemistry**. By Phillip D. Magnus. Pp. 27. No. 34: **The Synthesis of Insect Sex Pheromones**. By Clive A. Henrick. Pp. 45. No. 35: **Boraheterocycles via Cyclic Hydroboration**. By Herbert C. Brown and Ei-ichi Negishi. Pp. 27. No. 36: **Synthesis of Polyketide-Type Aromatic Natural Products by Biogenetically Modelled Routes**. By Thomas M. Harris and Constance M. Harris. Pp. 27. No. 37: **Ei Reaction of Sulphilimines and Related Compounds**. By Shigeru Oae and Naomichi Furukawa. Pp. 9. No. 38: **Strategies in Optical Resolutions**. By Samuel H. Wilen, André Collet and Jean Jacques. Pp. 12. No. 39: **The Diradical Mechanism for 1, 3-Dipolar Cycloadditions and Related Thermal Pericyclic Reactions**. By Raymond A. Firestone. Pp. 31. No. 40: **The Organic Photochemistry of Benzene-II**. By D. Bryce-Smith and A. Gilbert. (Oxford and New York: Pergamon Press, 1977 and 1978.) £4.95 each. [277]

International Union of Pure and Applied Chemistry: Applied Chemistry Division. Commission on Food Additives. Survey on Analytical Methods Available for the Estimation of Artificial Sweeteners in Food. Prepared for publication by B. D. Page and H. B. S. Conacher. Pp. 245–254. (Oxford and New York: Pergamon Press, 1978.) £3.30. [277]

List of University Institutions in the Commonwealth. Twenty-second edition. Pp. 35. (London: The Association of Commonwealth Universities, 36 Gordon Square, WC1, 1978.) Single copies *gratis*. [287]

The Electricity Council. Annual Report 1977–78. Pp. 24. (London: The Electricity Council, 39 Millbank, SW1, 1978.) [287]

Health and Safety Commission. Notification of Occupational Ill Health (Consultative Document). Pp. 9. (London: HMSO, 1978.) 50p net. [317]

Other countries—July

Deutsche Forschungsgemeinschaft. Jahresbericht Band 1: Tätigkeitsbericht 1977. Pp. 398. **Jahresbericht Band 2: Programme und Projekte 1977**. Pp. 856. (Bonn: Deutsche Forschungsgemeinschaft, Kennedyallee 40, 1978.) [147]

National Botanic Gardens of South Africa. Report 1974/1975–1976/1977. Pp. 34. (Kirstenbosch: National Botanic Gardens of South Africa, 1978.) [147]

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